BIOCHEMICAL, HAEMATOLOGICAL AND HISTOPATHOLOGICAL PARAMETERS OF MALE RATS ADMINISTERED AQUEOUS-METHANOL PULP EXTRACT OF *Azanza garckeana* AND GC-MS ANALYSIS OF THE FRUIT

BY

Idris Babatunde MOMODU
PG/LSC1102990

UNIVERSITY OF BENIN
BENIN-CITY

SEPTEMBER, 2021

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Idris Babatunde MOMODU
B.Sc. (Hons) Benin
PG/LSC1102990

A THESIS WRITTEN IN THE DEPARTMENT OF BIOCHEMISTRY AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (M.Sc) IN BIOCHEMISTRY OF THE UNIVERSITY OF BENIN, BENIN CITY.

SEPTEMBER, 2021

CERTIFICATION

We certify that this work was carried out by Mr. Idris Babatunde Momodu in the Department of Biochemistry, University of Benin, Benin City.

Prof. B.O Agoreyo **Date**
(Project Supervisor)

Prof. (Mrs.) K.E. Imafidon **Date**
(Head of Department)

External Examiner **Date**

CERTIFICATION OF THESIS

We the undersigned attest and declare that the thesis of Mr Idris Babatunde MOMODU titled: Biochemical, Haematological and Histopathological Parameters of Male Rats Administered Aqueous-Methanol Pulp Extract of *Azanza garckeana* and GC-MS Analysis of the Fruit has successfully passed the anti-plagiarism test and does not violate any copywrite regulations.

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Prof. B.O Agoreyo **Date**

(Project Supervisor)

______________________________ ______________________________

Prof. (Mrs.) K.E. Imafidon **Date**

(Head of Department)

______________________________ ______________________________

External Examiner **Date**

DEDICATION

This thesis work is dedicated to Allah the Almighty for His unending benevolence, my late father Alhaji Aliu Agbomere Momodu, my beloved mum and to all my amazing siblings.

ACKNOWLEDGEMENTS

My deepest gratitude goes to Allah the almighty for his blessings upon my life. This work will not be complete without expressing my immense gratitude to my supervisor Prof. (Mrs) B. O. Agoreyo for her patience, understanding, mentoring and immense contributions towards the success of this work. I will also like to express gratitude to the Head of Department, Prof. (Mrs) K. E. Imafidon, and to my esteem lecturers Prof (Mrs) E.S. Omoregie, Prof I. O. Onoagbe, Prof (Mrs) M. A. Adaikpoh, Prof N.P Okolie, Prof E. C. Onyeneke, Prof F. O. Obi, Prof E. A. C. Nwanze, Prof P.I. Campbell, Prof P. O and Dr (Mrs) R. I. Uadia, Prof C. C. Osunbor, Prof J. E. Orhue and Prof G. E. Eriyamremu for impacting in me.

To my late father, I know you will have been so proud with how far I have come, not to worry dad, the journey towards becoming a professor is still on. To my amazing mum, my deepest hope is that you live to enjoy the fruit of your labour. I am forever grateful mum. To my amazing siblings, words would not do justice to how much I love u all. To my Ameer Musa Iyere and my friend Sylvia Okungbowa, thank you both for being there when it felt so heavy and to others who contributed in one way or the other towards the realization of this work, thank you and God bless you all. To my MSc classmates, I appreciate you all for your love and contributions, it was an honour to serve.

Finally, to all individuals whose work were referenced in this study, I say thank you for your contribution to science.

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ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
CK	Creatine kinase
FBC	Full blood count
HDL	High density lipoprotein
HMGCoA	3-Hydroxy-3-methylglutarylCoA
LDL	Low density lipoprotein
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MPV	Mean platelet volume
NCI	National Cancer Institute
OECD	Organization for Economic Co-operation and Development
PDW	Platelet distribution width
RBC	Red blood cell
RDW	Red cell distribution width
WBC	White blood cell
LH	Luteinizing hormone
FSH	Follicle stimulating hormone
E2	Estradiol
PRO	Prolactin
PROG	Progesterone
TEST	Testosterone
TIC	Total ion chromatogram
RT	Retention time
GC-MS	Gas Chromatography Mass Spectrometry

ABSTRACT

Azanza garckeana is a tropical wild plant that is found in Africa. It produces edible fruits that are used as food or herbal medicine. The fruits are used for the treatment of anemia, malaria, liver problems and infertility. This study was aimed at determining the effect of the aqueous-methanol pulp extract of *Azanza garckeana* fruits on biochemical, haematological and histopathological parameters of male Wistar albino rats. The effect of the pulp extract on sex hormones was ascertained and the bioactive compounds present in the pulp and seeds of *Azanza garckeana* fruits were identified using gas chromatography-mass spectrometry (GC-MS) technique.

Twenty-four (24) male Wistar albino rats were used for the study and grouped into four groups with six rats per group; Group 1 (control) and three dose groups of 50, 300 and 2000 mg/kg body weight. The animals were acclimatized for two weeks. Those in the test groups were orally administered the aqueous-methanol pulp extract of *Azanza garckeana* fruits for twenty-eight (28) days. Analyses of biochemical, haematological and histopathological parameters, as well as sex hormone assessment were carried out using standard methods. Methanol and aqueous extracts of the pulverized pulp and seeds of the fruits were used for GC-MS analysis.

Results obtained from this study revealed no significant change ($p > 0.05$) between the control and test groups in the biochemical parameters assessed except for creatinine levels for 300 and 2000mg/kg bodyweight test groups, which significantly increased ($p < 0.05$) compared to control. The platelet crit, platelet count and RDW values were the only haematological parameters that were significantly different ($p < 0.05$) from the control. Histopathological study of the following organ; liver, kidney, heart, colon and testes showed no abnormalities in both test and control groups. A significant increase ($p < 0.05$) in the sex hormone progesterone at 300 and 2000mg/kg body weight test groups was observed. GC-MS analysis of the pulp and seeds of *Azanza garckeana* fruits revealed the presence of several bioactive compounds such as 5hydroxymethyl furfural, hexadecanoic acid, octadecane, heneicosane, heptacosane with varying medicinal properties. The consumption of *Azanza garckeana* fruits can be considered safe with no adverse effect. Moreover, identification of several bioactive compounds with medicinal properties that are present in the pulp and seeds of *Azanza garckeana* fruits gives credence to their ethnomedicinal uses.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

In the last decade, there has been a revival of interest in wild fruits as a way to meet people's food, nutritional and livelihood needs (Maroyi, 2012; Maroyi and Cheikh-Youssef, 2017). Since these fruits contain many micronutrients and phytochemicals that are essential for human nutrition and health. Evidence accumulated over time classifies a large proportion of these wild fruits as both food and medicines (Lako *et al.,* 2007; Lamien-Meda *et al.,* 2008). These wild edible fruits have medicinal properties that include antioxidant effects that counteract oxidative stress, making them important in the prevention of chronic illnesses including heart disease, cancer, diabetes, hypertension, stroke, and Alzheimer's disease (Lako *et al.*, 2007). Wild edible fruits have been discovered to have exceptional nutritional qualities, including an outstanding mineral source, fibre, vitamins, polyphenols, ascorbic acid, and fatty acids, all of which add flavour and colour to the diet (Glew *et al.,* 2005; Lamien-Meda *et al.,* 2008).

Some of these wild edible fruits are used in trado-medicine to treat a variety of illnesses. This probably could be due to their efficacy, availability and cost effectiveness of herbal medicines. They can also be used as food supplements to augment poor nutrition.

Some wild edible fruits have also been used to treat infertility, which is a major medical condition that affects about 10–15 percent of couples worldwide, as shown by a woman's failure to become pregnant after 12 months of unprotected intercourse (Zergers-Hochschild *et al*., 2009). Infertility in males is more prevalent compared to females (Ibrahim *et al.,* 2008). Male infertility has been linked to both environmental contamination and food choices in the last few decades, according to experimental proof and data analysis from numerous scientific studies (Chavarro, *et al.,* 2008; Ghanayem, *et al.,* 2010). Plants with fertility-enhancing and aphrodisiac properties have long been used in conventional practices. One of such plants used to enhance fertility in traditional practices is *Azanza garckeana*. *Azanza garckeana* is a wild tropical plant that has aphrodisiac proprieties and implicated as a remedy for infertility, and also used for the treatments of sexually transmitted diseases such as gonorrhoea. It is also used for treatment of other ailment like liver diseases in African countries such as Botswana, Kenya, Malawi and Nigeria (Ochokwu *et al.,* 2015; Dikko *et al.*, 2016).

Azanza garckeana **plant**

The plant *Azanza garckeana*, also known as 'goron tula' in northern Nigeria, is a member of the *Malvaeceae* family. It's a shrub with a height range of 3 to 15 meters. It is found in Central, East, South and West Africa. This plant has ethnomedicinal properties, and its bark, fruits, leaves, roots, and stems have been reported to have been used in the treatment or management of a variety of diseases throughout its distributional range. *Azanza garckeana* is used as medicinal plant in different ways in various African countries; in Nigeria and Botswana, the ripe fruits of *Azanza garckeana* has been implicated in the treatment of gonorrhoea, menstrual problem, anaemia, and infertility. It is also used as an aphrodisiac (Dikko *et al.*, 2016). In Kenya and Malawi, root decoctions, extracts from the leaf, stem, or ripe fruits of *Azanza garckeana* are used to treat infertility and liver diseases (Ochokwu *et al.,* 2015; Dikko *et al.,* 2016). In Sudan, the ripe fruit is consumed orally to treat anemia, while in Tanzania, a decoction made from the root of the *Azanza garckeana* tree is used to induce labor in expecting mothers (Augustino *et al.,* 2011). The fact that *Azanza garckeana* is consumed in these countries for various medicinal purposes does not guarantee safety. Since these ethnomedicinal claims of *Azanza garckeana* have not been scientifically proven, it is therefore important to ascertain its effects on biochemical, haematological and histopathological parameters, assess its effect on sex hormones, as well as identify the bioactive compounds present in the fruits of *Azanza garckeana*.

1.2 Justification of the study

The use of the pulp of *Azanza garckeana* plants for medicinal purposes in the treatment or management of various diseases (most especially in the treatment of infertility) calls for proper scientific investigation. This study therefore seeks to determine the effect of the pulp of *Azanza garckeana* fruits on biochemical, haematological and histopathological parameters, as well as its effect on sex hormones. In addition, there's also the need to confirm the medicinal properties of the fruit, hence gas chromatography-mass spectrometry (GC-MS) analysis will be used to identify the bioactive compounds present in the fruit of *Azanza garckeana* plant.

1.3 Aim and objectives of the study

The aim of this study is to determine the effect of the pulp of *Azanza garckeana* fruits on biochemical, haematological, and histopathological parameters of male Wistar albino rats. The effect of the pulp extract on sex hormones will also be ascertain and the bioactive compounds present in the pulp and seeds of *Azanza garckeana* fruits will be identified using gas chromatography-mass spectrometry (GC-MS) technique.

The objectives of this study are as follows;

1. To determine the effect of the pulp extract of the fruit on biochemical parameters (lipid profile, liver function and kidney function tests) of male Wistar albino rats.

2. To determine the effect of the pulp extract of the fruit on hematological parameters of male Wistar albino rats.
3. To determine the histopathological effect of the pulp extract of the fruit on liver, kidney, heart, colon and testes of male Wistar albino rats.
4. To determine the effect of the pulp extract of the fruit on sex hormones of male Wistar albino rats.
5. To ascertain the various bioactive compounds that are present in the pulverized pulp and seeds of the fruit by GC-MS technique.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Azanza garckeana*

Azanza garckeana is a vascular plant (also known as Tracheophyta) belonging to hibiscus family *Malvaceae*, and the sub family *Malvoideae. Azanza* is one of the genus of *Malvaceae* out of the estimated two hundred and forty-four (244) genera contain in the family *Malvaceae*. *Azanza garckeana* is closely related to the cotton plant (*Gossypium species*), which is also a member of the family *Malvaceae*.

2.1.1 Plant description

Azanza garckeana is an important wild tropical fruit plant found in Africa and commonly called 'goron tula' (kola of tula) in Northern Nigeria, snot apple in English and 'morajwa' (African chewing gum) in Botswana. The plant is also known as wild hibiscus (Orwa *et al.*, 2009; Ochokwu *et al.*, 2014). It is a member of the *Malvaceae* family which includes other well-known plants of economic importance such as okra, cotton, cacao (cocoa) and durian, which is native to Borneo and Sumatra. Durian is used to flavor a wide variety of savory and sweet desert in Southeast Asian cuisines**.**

Azanza garckeana is a deciduous shrub that grows to a height of three-fifteen meters, depending on the climate (Orwa *et al.,* 2009). There are many stems that make up the tree (Figure 2.1). The *Azanza garckeana* tree thrives in semi-arid environments (those with moderately low rainfall) with annual rainfall ranging from 250mm to 1270mm. Flowering occurs during the rainy season, which lasts from May to October, while fruit ripening occurs during dry season between November to April (Orwa *et al.,* 2009; Ochokwu *et al.*, 2014).

The leaves of *Azanza garckeana* tree are distinctively simple, alternate and roundish (plate 2.2) with 3 to 5 lobes. The young leaves of *Azanza garckeana* tree are velvety and bronze in colour (Mojeremene and Tshwenyane, 2004). The fruits of *Azanza garckeana* is spherical and have woody pulp. The unripe fruits are green in colour and turn brown when ripe. The pulp of *Azanza garckeana* fruits are divided into five (5) segments or capsules (plate 2.3) with each segment or capsule containing a seed that is hemispherical in shape and covered with brownish woolly floss (plate 2.4) (Orwa *et al.,* 2009).

2.1.2 Distribution of *Azanza garckeana*

The presence of *Azanza garckeana* has been recorded in Central Africa (DR Congo), East Africa (Burundi, Kenya, Tanzania), South Africa (Zimbabwe, Botswana, Malawi, Namibia, Zambia), South East Africa (Mozambique), West Africa (Nigeria), and North Africa (Sudan). Here in Nigeria, *Azanza garckeana* is located in Tula region of Kaltungo Local Government Area of Gombe State and in Michika of Adamawa state.

2.1.3 Dietary Uses of *Azanza garckeana*

The pulp of the ripe fruits of *Azanza garckeana* is edible and widely consumed throughout the distribution range. They are used as food additives to enhance food taste and also made into porridge (Maroyi and Cheikh-youssef, 2017).

2.1.4 Ethnomedicinal uses of *Azanza garckeana*

A variety of pharmacological activities of *Azanza garckeana's* tree bark, leaves, and fruit pulp have been published in scientific literature, supporting some of the plant's ethnomedicinal uses. Antibacterial, antihyperglycemic, antimalarial, antioxidant, and iron absorption are among the recorded activities.

Plate 2.1: The tree of *Azanza garckeana*

Plate 2.2: Tree of *Azanza garckeana* with leaves and fruit (Mojeremane and Tshwenyane, 2004)

Plate 2.3 Fruits of *Azanza garckeana*

Plate 2.4 Seeds of *Azanza garckeana*

2.1.4.1 Antibacterial Activity

Enterococcus species are gram positive bacteria and facultative anaerobes, to which *Enterococcus faecalis* and *Enterococcus faecium* are the most prevalent. These prevalent species of *Enterococcus* are commonly found in humans and are known to be causative agents for a variety of infections (Ryan and Ray, 2004). Mutindi (2014), reported the antibacterial activities of *Azanza garckeana* crude root extract and pure compounds isolated from the plant's roots against these *Enterococcus species*. *(Enterococcus faecalis* and *Enterococcus faecium*). The antibacterial activity of the compound Gossypol 1 found in the roots of this plant has also been confirmed against the bacteria *Staphylococcus aureus* and *Enterococcus faecium* (Masila *et al.*, 2015).

2.1.4.2 Antihyperglycemic Activity

Amuri *et al.,* (2017) found that aqueous leaf extracts of *Azanza garckeana* have hypoglycemic and antihyperglycemic properties. This finding may support the traditional use of *Azanza garckeana* leaf as herbal medicine for diabetes (Ahmed *et al.*, 2016).

2.1.4.3 Antimalarial

Antimalarial evaluations were carried out using crude extracts of *Azanza garckeana* and they were reported to have weak antimalarial activities (Maroyi and Cheikh-youssef, 2017).

2.1.4.4 Antioxidant

Azanza garckeana methanol stem bark extract has been shown to have antioxidant properties (Mshelia *et al*., .2016).

2.1.4.5 Iron absorption

The stimulating iron absorption properties of *Azanza garckeana* extract may justify its use in the treatment of iron deficiency anemia (Ahmed *et al.,* 2016).

2.2 Infertility

The World Health Organization defines infertility as a couple's failure to conceive after a year of uncontrolled intercourse. Infertility affects around 15% of couples around the world (Agarwal *et al.*, 2015). Male disorders account for 30–50% of these cases of infertility (Sharlip *et al.*, 2002). In about 30 to 40 percent of these infertility cases in males, the problem is with the testes, which are the glands that produce sperm and testosterone (the primary male sex hormone). Infections such as mumps, cancer treatments such as radiation or chemotherapy, trauma, or surgery, and environmental exposure to toxicants such as cadmium can all cause damage to the testes (Hollund, *et al*., 2000). Varicocele, or enlargement of the veins around the testes, may also result in low sperm output and poor sperm quality. The expanded veins overheat the testes, resulting in low sperm output and poor sperm quality. For optimal sperm development, maturity, and work, the testes need a body temperature that is lower than our core body temperature. Since the scrotum's body heat is around five degrees lower than that of the abdomen or pelvis, overheating of the testes may occur when the veins around the testes become swollen, as in varicocele. As a result, sperm production and function are reduced, resulting in a lower fertility capacity (Baazeem *et al.,* 2011). Hormone deficiency can also cause infertility. The sex hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH) stimulate the gonads and are necessary in both males and females. They are produced in the pituitary gland, which is located in the brain. LH and FSH activate the ovaries to produce eggs and the reproductive organs to produce sex hormones in males and females respectively (testosterone and estrogen) (Yakubu *et al*., 2007). Low LH and FSH levels can result in a condition known as hypogonadotropic hypogonadism that results from gonadal failure due to abnormal pituitary gonadotropin levels (Basaria, 2014). Testicular obstruction, low semen volume, sperm agglutination, idiopathic infertility, ejaculatory dysfunction, irregular viscosity, endocrine disorder, high sperm density, and congenital defects are some of the other causes of infertility (Bayasgalan *et al*., 2004). Male infertility treatment is determined by the cause. If testicular obstruction is the cause of infertility, surgery may be necessary. Gonadotropin therapy is usually used in the treatment of male infertility that occurs due to hypogonadotrophic hypogonadism. Maintaining a healthy lifestyle, exercising often, eating a healthy diet, no smoking or use of recreational drugs, and continuing care for any chronic condition are all recommended and helpful ways to increase the odds of successful infertility treatment.

2.3 Biochemical Parameters

2.3.1 Lipid Profile

Lipid profile is a collection of blood tests that is used as a first step in screening for lipid abnormalities. Total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides are the most common.

2.3.1.1. Total Cholesterol

Cholesterol is a modified steroid of high molecular weight that possesses the cyclo-pentanophenanthrene structure and is found in animal tissues only. It can either occur as free sterol or esterified with long chain fatty acid. Over 80 % of cholesterol needed by the body is synthesized endogenously in the liver and intestine via β-hydroxyl β-methylglutaryl-CoA (HMGCoA) reductase activity. The other 10-20 % needed in the body are assimilated via the intestine from exogenous sources. Cholesterol is a building block for bile acid, steroid hormones and fat-soluble vitamin D synthesis. Cholesterol is also a necessary part of the membranes of animal cells. Cholesterol measurements are used to diagnose and treat lipid lipoprotein metabolic disorders including hypercholesterolemia, a condition where there is increased levels of serum cholesterol and this condition has been connected with coronary heart infection and atherosclerosis. Cholesterol homeostasis is also essential for testicular functions including steroidogenesis and the production of sex hormones. Changes in plasma cholesterol concentration may have an effect on reproductive function, resulting in male infertility. In patients with hyperlipidemia or metabolic syndrome, the correlation between lipid homeostasis and reproductive function is clearly visible (Lauriane *et al.*, 2018).

Total cholesterol is a measurement of the total amount of cholesterol in the blood at any given time, and includes both low density lipoprotein (LDL) and high density lipoprotein (HDL). LDL being the primary cause of cholesterol build-up and artery blockage, while HDL aids in the removal of cholesterol from the arteries. The term good or bad cholesterol relates to the ratio of HDL to LDL. A high ratio of HDL to LDL is desirable, while a low HDL to LDL ratio is undesirable.

2.3.1.2 HDL-Cholesterol (HDL-C)

High density lipoproteins are one of the five (5) main groups of serum lipoproteins and play essential roles in removing cholesterol from tissues and transporting it to the liver. Cholesterol is transferred to the liver, where it is converted into bile acids, which aid fat digestion. HDL-Cholesterol is made up of a variety of different particles, including lipid, apolipoprotein, and cholesterol. These heterogeneous particles differ with regards to size, and composition. HDL is termed the good cholesterol and it is desirable in higher level compared to LDL. Reduced plasma HDL-Cholesterol concentrations are associated with an increased risk of atherosclerosis, myocardial infarction, and cerebrovascular injury (stroke) (Friedman and Young, 2001). HDL-Cholesterol concentrations differ greatly depending on age and gender. Values ≤40 mg/dL (1.00

mmol/L) is considered as low HDL level, while ≥ 60 mg/dL (1.56 mmol/L) is said to be high level and has protective value against cardiovascular disease (NCEP, 2001).

2.3.1.3 LDL-Cholesterol (LDL-C)

Low density lipoproteins (LDL) is a type of lipoprotein. It is often referred to as bad cholesterol because high LDL levels cause a build-up of cholesterol in the arteries. LDL-C, along with other substances (such as fat, calcium) combine to form plaque. The plaque is what build-up in the arteries causing an obstruction in blood flow that leads to a condition called atherosclerosis. Diet, weight, lack of physical activity, smoking, genetics and other medical conditions can affect the LDL level. Low LDL- cholesterol level is preferable, as a high LDL level is risk indicator of coronary artery disease and related problem such as heart disease. Values < 100 mg/dl are considered as optimal

2.3.1.4 Triacylglycerol

The main storage form of fatty acid in human adipose tissue is triacylglycerol (TAG), which is an ester derived from glycerol and three fatty acids. TAG is also found in blood lipids, allowing for bi-directional adipose fat and blood glucose transfer from the liver. TAG estimations are used in the diagnosis and treatment of lipid metabolism diseases. Normal serum level of TAG is ≤ 150 mg/dL and anything higher is suggestive of hypertriglyceridemia.

2.3.2 Liver Function Tests

Scientifically, biochemical changes serve as necessary tools in the evaluation, identification and characterization of liver pathology. Several biomarkers like liver transaminases (alanine aminotransferase (ALT), aspartate aminotransferase (AST)), alkaline phosphatase (ALP), and bilirubin are useful in the diagnosis of liver damage or injury (Singh *et al.*, 2011). Increase in the activities of the above named enzymes in the serum are considered as vital indicator of liver damage. Changes also in blood concentrations of substances such as bilirubin that are processed normally in the liver is used as a measure to evaluate the function of the liver. Bilirubin levels that are higher than normal can suggest a liver or bile duct problem. A rise in AST, ALT, and ALP levels above average, combined with a rise in bilirubin levels that are more than double the normal is a warning sign of hepatotoxicity (Obi *et al.*, 2004).

2.3.2.1 Alanine Aminotransferase (ALT)

Alanine aminotransferase (ALT) also called alanine transaminase was initially known as serum glutamic pyruvic transferase (SGPT). The enzyme was first characterized in mid-1950. The enzyme is present in the liver, muscle and heart but greater proportion is found in the liver compared to the muscle and the heart. ALT catalyses the transamination reaction, in which - oxoglutarate (ketoglutarate) and L-alanine are converted to L-glutamate and Pyruvate. ALT levels in the blood are normally low, but when the liver is weakened, ALT is released into the bloodstream, thereby raising the level. The presence of liver disease is often indicated by significantly elevated ALT (SGPT) levels. To track or diagnose liver disease, ALT and aspartate transaminase (AST) are used together as part of a comprehensive metabolic examination. The AST/ALT ratio is measured and used to differentiate between the various causes of liver damage as well as to aid in the recognition of heart or muscle injury. To help assess the type of liver disease present, ALT values are compared to other test results such as alkaline phosphatase (ALP), AST, total protein, albumin to globulin ratio, and bilirubin. The ALT range considered to be normal is between 7–56 U/ L and elevations above this levels may indicate liver cell injury. Patients with hepatic disorders such as ischemic liver injury (shock liver), viral hepatitis, and other liver damage are more likely to have noticeable increase of ALT values greater than 500 U/L (Gowda *et al.,* 2009).

2.3.2.2 Aspartate Aminotransferase (AST)

The enzyme aspartate aminotransferase (AST) catalyses transamination reaction. AST catalyse the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate. The enzyme is found primarily in striated muscle, the myocardium, and the liver. In human tissues, the enzyme AST exists as two distinct isoenzymes, one in the cytoplasm and the other in the mitochondria. The measurement of AST isoenymes in human serum is helpful in determining the extent of damage to these organs. An injury to liver or muscle cells causes a release of AST into the blood. Standard serum AST levels ranges between 0 to 35 U/L. The AST values may be amplified in the range of 10 to 20 times the normal values in cases of serious liver damage (Huang *et al.,* 2006).

2.3.2.3 Alkaline Phosphatase

The enzyme alkaline phosphatase (ALP) is present in the placenta, epithelial mucosa of the small intestine, liver, proximal convoluted tubule of the kidney, and bone. However, the liver and bones are the main sources of serum ALP activity. The standard range for serum ALP is 41 to 133 U/L. Liver disease, bile duct obstruction, gall bladder disease, and bone disorders are the most common causes of elevated ALP levels in the blood. ALP is considered non-specific for determining liver damage, thus to differentiate liver diseases from bony disorders, gamma glutamyl transferase level is determined as it is increased only in cholestatic disorders (a condition in which the flow of bile from the liver stops or slows and is caused by liver infection) and not in bone diseases (Gowda *et al.,* 2009).

2.3.2.4 Serum Bilirubin

Bilirubin is a yellow compound that is produced as a catabolic product of haemoglobin in the reticuloendothelial system of the liver, spleen, and bone marrow. Unconjugated bilirubin leaves the synthesis site and is transferred to the liver. The unconjugated bilirubin is transported in the blood plasma bounded to albumin. In the liver, the unconjugated bilirubin is converted into its conjugated forms bilirubin mono and di-glucuronides by the enzyme uridine diphosphate glucuronyl transferase. The conjugated bilirubin is then secreted into bile by an ATP- dependent transporter. Bilirubin is excreted in bile and urine. The range of total bilirubin (unconjugated and conjugated) for normal subject varies between 2 – 21 μmol/L. The normal levels of the unconjugated bilirubin are <12 μmol/L where as that of conjugated bilirubin is <8 μmol/L. Serum bilirubin levels that are abnormally high could mean that the red blood cells are breaking down too quickly or that the liver is failing to properly break down waste (Gowda *et al.*, 2009).

2.3.2.5 Albumin

Albumin is a major protein found in blood serum and is synthesized by the liver. Albumin executes several roles in the body, some of which include transportation of different substances such as drugs, hormones and vitamins through the body, prevention of fluid leakage from blood vessels and nourishment of tissues. Reduction in Albumin levels (hypoalbuminaemia) in the blood may signify kidney damage, shock, liver damage, severe inflammation or malnourishment. Hyperalbuminemia (increase in albumin level) however have little diagnostic significance but may be an indication of dehydration. Scientists may use albumin values to assess or test for lupus-related kidney or liver disease. Lupus is an autoimmune disease in which the immune system attacks its own tissues (Walker *et al.*, 1990).

2.3.2.6 Evaluation of Total Protein

The total protein level in the body can be used to distinguish between patients with hepatic damage and healthy people (Thapa and Walia, 2007). Total protein refers to the total sum of two forms of proteins found in the blood: albumin and globulin, both of which are synthesized in the liver. Hence, total protein test estimates the joint concentrations of both proteins (globulin and albumin) in the blood. A normal total protein values ranges from 6.0 to 8.3 g/dl. A patient's total protein level may also give information about renal damage apart from liver damage. If an individual's total protein reveals an anomalous value, a kidney or liver function tests are recommended. Nonetheless, a sharp decline in albumin to globulin (A/G) ratio may signify hepatic damage. Hyperproteinaemia (increase in protein) can be caused by dehydration or as a result of increase in the concentration of specific proteins (immunoglobulins in chronic infection and myeloma) (Friedman and Young, 2001), while hypoproteinaemia (decrease in protein) may be caused by low haematocrit resulting from increased plasma volume (hemodilution), severe malnutrition, chronic liver disease or by an excessive protein loss due to chronic kidney disease (Friedman and Young, 2001).

2.3.3 Kidney Function Tests

Creatinine, urea and electrolytes are indicators or markers of kidney function. These indicators are expedient in assessing normal kidney function and are useful for indication of the rate of glomerular filtration, as well as the functions of the tubules. A remarkable increase or decrease in the levels of these indicators or markers signifies kidney dysfunction (Gowda *et al.*, 2010).

2.3.3.1. Creatinine

Creatinine is a catabolic breakdown product that is synthesized from creatine and phosphocreatine in skeletal muscles and maybe defined as nitrogenous waste product. The kidneys filter nearly all of it from the blood and excrete it in urine. The amount of creatinine in the blood is measured with a creatinine test. The estimation of serum creatinine levels has been established as the most popularly used screening test to determine kidney failure (Swedko *et al.,* 2003). The reference interval for creatinine level is 0.6 -1.3 mg/dl (Lewis *et al.,* 2014). Levels higher than the reference interval may be an indication of renal dysfunction or failure. Chronic renal failure is characterized by an ultimate reduction in the removal of creatinine by both the tubules and glomeruli (Gowda *et al.,* 2010).

2.3.3.2 Urea (Blood Urea Nitrogen)

The blood urea nitrogen (BUN) test determines how much nitrogen is present in the bloodstream as a result of the waste product urea. It is one of the oldest nephrology biomarkers or indicators (Waikar and Bonventre, 2006). Urea is a by-product of the degradation of proteins. It is synthesized in the liver and excreted in the urine. A BUN test is carried out to ascertain if the kidney is functioning properly. When the kidneys are unable to extract urea from the blood, the blood urea level rises. Normal reference range of BUN in the blood is 2.1-7.1 mmol/L or 6-20 mg/dL (Lewis *et al.,* 2014).

2.3.3.3 Electrolytes

Electrolytes are salts and minerals that are present in the blood, such as sodium, potassium, chloride, and bicarbonate. Electrolytes aid in the transport of nutrients into the body's cells and waste out of the body's cells. They also help to regulate the body's acid/base balance, conduct electrical impulses, and maintain water balance. Test for electrolyte can help to determine electrolyte imbalance in the body. The tests for electrolytes shows the measurement of sodium, chloride, bicarbonate, and potassium in the blood. Potassium is the most reliable electrolyte marker of renal failure, according to research. Kidney failure results in increased plasma potassium (hyperkalemia) due to a combination of decreased filtration and secretion of potassium in the distal tubules. Hyperkalemia is a life-threatening complication of renal failure (Wu, 2006).

2.4 Haematology

The study of the numbers and morphology of the cellular components of the blood, namely red blood cells (erythrocytes), white blood cells (leucocytes), and platelets (thrombocytes) is referred to as haematology. The status of exposed animals to toxicants and other conditions such as disease is reflected in their blood (Olafedehan *et al.*, 2010; Togun *et al.*, 2007). The results obtained from carrying out tests on haematological parameters are important in the diagnosis and monitoring of diseases. One of the most important and recurrent laboratory tests used for haematological studies in diagnosis of disease affecting the blood is the complete or full blood count (FBC). The FBC tests consist of 13–19 parameters. The clinical use of the FBC test is elaborate; an increased or decreased in total white blood cell count (WBC) may be as a result of irregular bone marrow pathology. Increase in total white blood cells with a high lymphocyte count or increase circulatory neutrophils could indicate the existence of a viral or microbial infection. The red blood cell indices values can be used to infer the aetiology of anaemia. The platelet count and size can be used to assess the bone marrow's platelet formation activity. A decrease or increase in platelet count can also point to liver infection or hemostatic disorder (Osei-Bimpong *et al.*, 2012).

2.4.1 Haematological Parameters

Haematological parameters, which consist of 13–19 parameters can be grouped as red blood cell indices (red blood cell count (RBC), haemoglobin count (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), packed cell volume (PCV)), white blood cell differential, Platelet evaluation (platelet count (Pt), mean platelet volume (MPV), platelet distribution width (PDW)

2.4.1.1 Red Blood Cells Indices

A total or full blood count (FBC) examination includes red blood cell indices such as Red blood cell count (RBC), haemoglobin count (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin, (MCH), mean corpuscular haemoglobin concentration (MCHC) and the red cell distribution width (RDW). These indices can be used to diagnose and identify anaemias, a disorder in which there aren't enough red blood cells in the body. The MCV are measured in femtoliter and the normal range is 80-100 femtoliter. The normal range for MCH is 27-31 pg/cell. The normal range for MCHC is 32-36 g/ dL and the normal percentage range for RDW is 11.5-14.5 %.

2.4.1.2 White Blood Cell Differential

White blood cells (WBCs) or leukocytes are essential components of the immune system and they include various sub-populations such as basophil, granulocytes, monocytes, lymphocytes, neutrophil and eosinophil. In the bone marrow, leukocytes are produced, while lymphocytes are produced in the lymphoid tissues. (thymus and the red bone marrow). White cell differential is a laboratory procedure used to assess the forms and number of white blood cells in a blood sample as part of a full blood count (FBC). These findings are presented as percentages and absolute values, and they are compared to a set of benchmarks. The reference range for WBC in adult is 3.6-10.6 x 10^9/L. Changes in the number of white blood cells can help diagnose a variety of illnesses, including infectious, bacterial, parasitic, and blood disorders.

2.4.1.3 Platelet Evaluation

Platelets, also known as thrombocytes are blood cells that lack nucleus and play important role in maintaining haemostasis (Assinger, 2014). Platelet clump together in response to bleeding from a blood vessel injury, causing a blood clot to form. The fragmentation of the cytoplasm of megakaryocytes in the bone marrow gives rise to platelets (Assinger, 2014). Platelet count (Pt), mean platelet volume (MPV), and platelet distribution width (PDW) are all platelet parameters used in an automated full blood count. The reference range for platelet is 150-450 x 10^9 /L.

2.5 Histopathology

Histopathology examination is a branch of histology that deals with the identification and analysis of diseased tissue under the microscope. Accurate diagnosis of cancer and other diseases often necessitates histopathological analysis of tissue samples, it is a vital part of anatomical pathology and surgical pathology (Rosai, 2007).

2.6 Sex Hormones

Hormones are chemical messengers produced and released into the bloodstream by the endocrine glands, and they aid in the regulation of many bodily functions such as appetite, sleep, and development. Hormones that play an important role in sexual growth and reproduction are known as sex hormones. Both males and females depend on sex hormones for a variety of bodily functions. They are involved in sexual development during puberty, reproduction, body fat distribution and promotion of hair growth. A sex hormone imbalance can result in changes in sexual desire as well as health issues like infertility. Androgens (testosterone, which is the primary reproductive hormone in males), estrogens, follicle stimulating hormone, luteinizing hormone, and prolactin are examples of these hormones.

2.6.1 Androgen (Testosterone)

Androgens are a class of hormones that influence male characteristics and sexual activity. Androgens can be found in both men and women. Testosterone and androstenedione are the two main androgens. Males' main sex hormone is testosterone. Testosterone is produced mainly by the leydig cells of the testis in males, and to a lesser degree by the ovaries in females. Testosterone is involved in the development of male sexual traits, sperm formation, and sex drive (libido). Furthermore, testosterone aids in the prevention of low bone density, which is seen in osteoporosis (Tuck and Francis, 2009). Its measurements are helpful in evaluating functional activity of the testes or the ovaries. Virilization, polycystic ovaries, ovarian tumors, adrenal tumors, and adrenal hyperplasia are all conditions under which elevated levels of testosterone are present in women. High testosterone levels have been linked to hypothalamic pituitary unit disorders, testicular cancers, congenital adrenal hyperplasia, and prostate cancer in males. Low testosterone levels cause males' libido and erections to decrease, as well as more aches and pains in the bones and joints than normal. Low testosterone levels can be seen in patients suffering from hypopituitarism and testicular feminization. In general, the normal range in males is about 270 ng/dL-1070 ng/dL with an average of 679 ng/dL.

2.6.2 Estrogen (Estradiol)

Estrogen is a hormone that has many functions in the body. It aids the growth and control of the female reproductive system, as well as secondary sex characteristics in females. Estrogen controls certain reproductive functions in men, which are essential for sperm maturation and may be required for a healthy libido (Hill *et al.*, 2004). Three main endogenous estrogens of estrogenic hormonal activity are present in the body. Estrone, estradiol (E2), and estriol are the three hormones (E3). Estradiol is the most powerful and prevalent, released mostly by the ovary and placenta, with minor quantities produced by the adrenal cortex and male testes. Males have considerably lower levels of estrogen than females. Patients of feminizing syndromes, bloated male breast tissue (gynaecomastia) and testicular tumors have been shown to have elevated estradiol levels. In general, the normal range in males is about 10-40 pg/ml.

2.6.3 Progesterone

Progesterone is a steroid that belongs to the C21 group. It is an endogenous steroid that belongs to the progestogen family of steroid hormones. Progesterone is produced primarily by the adrenals and ovaries, as well as the placenta during pregnancy. The reproductive organs play the most important role in progesterone's function. Progesterone is an essential intermediate in the synthesis of other endogenous steroids in men, such as androgens and corticosteroids. The normal range in adult males is ≤ 0.2 ng/ml.

2.6.4 Follicle Stimulating Hormones (FSH)

FSH is a glycoprotein synthesized by the anterior pituitary gland. FSH is active in the early stages of spermatogenesis in males and promotes seminiferous tubule, testicular development, and testicular growth. FSH promotes follicular development and trains ovarian follicles for luteinizing hormone action in females (LH). Lack or insufficiency of this hormone can cause infertility or subfertility in both men and women. Following menopause, castration, and early ovarian failure, FSH levels rise. FSH levels are typically higher in males with low sperm output. Testicular tumors tend to lower serum FSH levels. Primary testicular failure and hyperthyroidism can also cause high FSH levels in men.

2.6.4 Luteinizing Hormone

The anterior pituitary gland produces luteinizing hormone (LH) in both males and females in response to gonadotropin releasing hormone (Gn-RH) (George and Kaiser, 2018). LH is a 30,000-dalton glycoprotein. It is made up of two non-covalently related subunits, the alpha and beta. In females, LH promotes ovulation and the synthesis of ovarian steroid (estrogen). LH regulates the release of testosterone by leydig cells in men. LH levels are higher in the latter phase of the menstrual cycle, in people who have gonad problems, and in women who are approaching menopause.

2.7 Gas Chromatography- Mass Spectrometry

Gas chromatography (GC) is a method used in analytical chemistry to separate and analyse molecules that can be vaporized without decomposition. Gas chromatography, like all other forms of chromatography, consists of a mobile phase and a stationary phase. A gas is the mobile phase, and an immobile high molecular weight liquid is the stationary phase, which is deposited on or chemically bound to the inner walls of a long capillary conduit. The gas chromatograph (GC) can be used to distinguish volatile and semi-volatile compounds with considerable precision, but it cannot be used to classify them. As a result, a technique known as Gas Chromatography-Mass Spectrometry (GC-

MS) is used to identify the compounds. This can be derived by coupling a GC equipment to a mass spectrometer (Sloan *et al.,* 2001).

Mass spectrometer (MS) can help to provide detailed structural information on most compounds thus enabling their identification (Amirav *et al.,* 2008; Quazi *et al.,* 2011). The flow rate from the capillary column is guided into the MS's ionization chamber in GC-MS. The Ion Trap Mass Detector (ITD) is a relatively popular mass detector used in GC-MS (Figure 2.1). Ions are produced from the eluted sample in this instrument through electron impact or chemical ionization and deposited in a radio frequency field. The ions are then expelled from the storage area and sent to an electron multiplier detector. The ejection is monitored to allow scanning based on mass-to-charge ratio. Hundreds of components in natural and biological samples have been identified using GC-MS instruments (Amirav *et al.,* 2008; Quazi *et al.,* 2011).

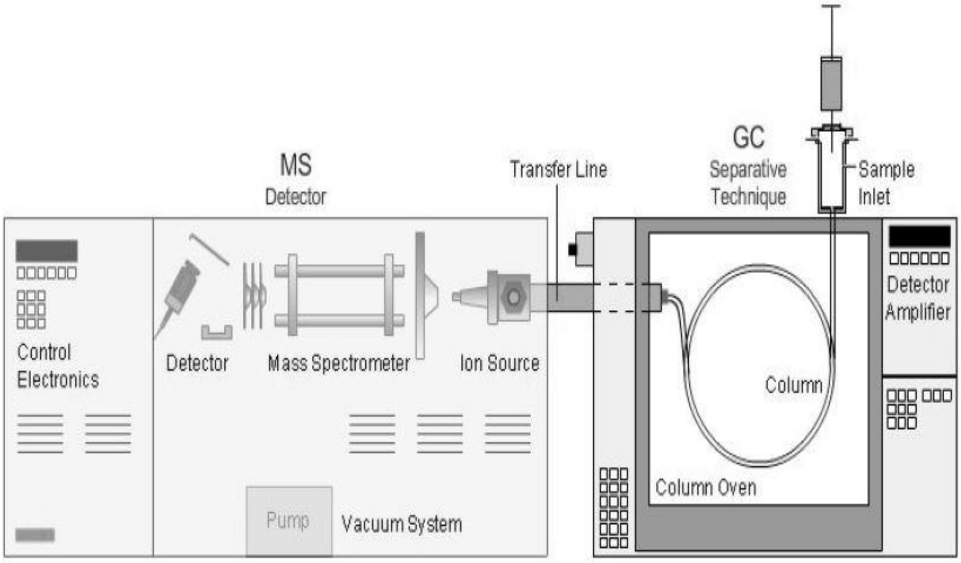


Fig. 2.1: Diagram of a GC-MS System (Quazi *et al.*, 2011)

CHAPTER THREE

MATERIALS AND METHOD

3.1 Materials

3.1.1 Equipment

Electric oven (Genlabwidness, England), Weighing balance (Mettler analytical balance, USA), UV spectrophotometer (Unico, China), Glass wares (Pyrex, England), Water bath (TT42D Multipurpose use, Techmel and Techmel, USA), Micropipette (Microplux, USA), Stirrer, Syringe, Gas chromatography (Agilent Technologies, Santa Clara. CA, USA), Centrifuge (Model 80-2, Harris, England), Muslin cloth, ELISA reader (Stat Fax 4700, Awareness Technologies, USA).

3.1.2 Chemicals and Reagents

Analytical grade chemicals and reagents were used. They were obtained from standard commercial suppliers. 98 % methanol, distilled water, chloroform Triglyceride kit, total cholesterol kit, high density lipoprotein kit, low density lipoprotein, aspartate transaminase kit, alanine transaminase kit, alkaline phosphatase kit, total protein kit, total bilirubin kit, albumin kit, creatinine kit, urea kit were products of Randox Laboratories, UK. and Sex hormones ELISA kit were obtained from Calbiotech, USA.

3.2 Sample collection and identification

Fresh *Azanza garckeana* leaves and fruits were collected from Tula in Kaltungo Local Government Area (LGA) of Gombe State, Nigeria on the 26th September 2019. The plant sections were then identified and authenticated at the Forest Research Institute of Nigeria (FRIN), Ibadan. A voucher specimen deposited in their reference with the registration number FHI-112621.

3.2.1 Preparation of pulverized samples

To extract moisture content, the seeds and pulp of *Azanza garckeana* fruits were separated and oven dried at 60°C. After which, the pulp and the seeds were crushed into powder using mortar and pestle**.** The pulverized pulp and seeds were kept in an airtight jar at room temperature until they were extracted.

3.2.2 Preparation of Aqueous-Methanol Extract

The pulverized pulp weighing 3500 g was soaked for 72 hours in a mixture of 80% methanol and 20% water (v/v) and stirred every day. A muslin cloth was used to filter the mixture. To remove the methanol, the filtrate was condensed using a rotary evaporator set at 60°C and then freeze dried to remove the moisture. Prior to administration, the aqueous-methanol extract was held in the refrigerator at 4°C.

3.3 Experimental Animals

Adult male Wistar albino rats were obtained from a local breeder in University of Benin, Benin City, Edo State and housed in the Animal house of the Department of Biochemistry, Faculty of Life Sciences, University of Benin.

3.3.1 Experimental Design

This study used a total of 24 male Wistar albino rats. The experimental animals weighed between 150-198 g and were grouped into four (4) groups (Groups 1-4), with six (6) animals in each group. The control group was number one, and the test groups were numbered two through four. The animals were held in clean, disinfected wooden cages for 14 days prior to extract administration. Pelletized feed was given to them. The feed and water were given *ad libitum*.

Group 1: Control group containing six (6) animals were administered the vehicle only, which is 1 ml distilled water.

Plate 3.1: The pulp of *Azanza garckeana* after seed removal

Plate 3.2: The pulp of *Azanza garckeana* after pulverization using mortar and pestle

Plate 3.3: Seeds of *Azanza garckeana*

Plate 3.4: The seeds of *Azanza garckeana* after pulverization using mortar and pestle

Group 2: The aqueous-methanol pulp extract of *Azanza garckeana* was given orally to the six (6) experimental animals in this group at 50 mg/kg body weight. Calculated gram of the extract that was administered to each rat was dissolved in 1 ml of distilled water.

Group 3: The aqueous-methanol pulp extract of *Azanza garckeana* was given orally to the six (6) experimental animals in this group at 300 mg/kg body weight. Aliquot of 1 ml distilled water containing the calculated gram of the extract based on bodyweight of each rat was administered.

Group 4: The aqueous-methanol pulp extract of *Azanza garckeana* was given orally to the six (6) experimental animals in this group at 2000 mg/kg body weight. Aliquot of 1 ml distilled water containing the calculated gram of the extract based on bodyweight of each rat was administered.

Following the administration of the aqueous-methanol pulp extract to the different test groups, the rats were examined for 2 hours for changes in skin, hair, eyes, behavioural habits, tremors, salivation, diarrhoea, sleep, coma, and mortality, and then regularly for 28 days for changes in skin, fur, eyes, tremors, salivation, diarrhoea, sleep, coma, and mortality. During the analysis, food intake and weight changes were also observed on a regular basis. Prior to sacrifice, the animals were fasted overnight (12 hours) at the conclusion of the experimental period. The rats were anesthetized with chloroform, and blood samples were taken from the abdominal aorta and heart. Blood samples were used to assay for these parameters (biochemical, haematological and sex hormones). The heart, liver, kidney, colon and the testes were harvested and used for histopathological examination.

3.4 Biochemical Parameters

The blood samples for biochemical parameter were collected in universal containers (non-heparinised) and allowed to stand for 10 minutes. The blood samples were then centrifuged at 6000 rpm for 10 min to obtain serum that was used to determine the following biochemical parameters, total cholesterol, Low Density Lipoproteins (LDL), High Density Lipoproteins (HDL), Triglycerides (TG), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Total Protein, Albumin, Total Bilirubin, Conjugated Bilirubin, Urea, Creatinine were analysed using Randox diagnostic kits. Plasma electrolytes (Na+, K+, Cl- and bicarbonate) were analysed using automated analyser.

3.4.1 Lipid Profile Tests

3.4.1.1 Determination of HDL- Cholesterol

This was determined by the method of Lopes-Virella *et al*. (1977).

Principle

Low density lipoprotein (LDL and VLDL) and chylomicron fractions in the serum interact with phosphotungstic acid in the presence of magnesium ions and are quantitatively precipitated. The HDL-cholesterol (high density lipoprotein) fraction persists in the supernatant, and the cholesterol pap process is used to assess it.

Procedure

The blank, normal, and tests test tubes were filled with 100 µL of purified water, standard solution, and serum samples, respectively. The cholesterol Reagent (which contains Phosphotungstic acid and magnesium chloride) was then applied to each test tube in 1 mL increments. Each tube's contents were mixed and incubated for 10 minutes at 25°C. The absorbance of the sample (A_{sample}) and of the standard ($A_{standard}$) were read against the reagent blank at 500 nm within 60 minutes. The test was carried out in triplicate. The HDL-cholesterol concentration in the serum was then calculated with the equation below:

$$\text{HDL-cholesterol CONCENTRATION (mg/dl)} = \frac{\text{Absorbance of sample x concentration of standard}}{\text{Absorbance of standard}}$$

3.4.1.2 Determination of total cholesterol

This was determined by the method of Reiser *et al.* (1985).

Principle

After enzymatic hydrolysis and oxidation, the cholesterol is measured. In the presence of phenol and peroxidase, the oxidation agent (hydrogen peroxide) interacts with 4-aminoantipyrine to produce the indicator quinoneimine dye, whose intensity is proportional to the amount of total cholesterol present in the sample.

Cholesterol ester + H_2O $\longrightarrow$ Cholesterol + Fatty acids

Cholesterol + O_2 $\longrightarrow$ Cholesterene-3-one + H_2O_2

$2H_2O_2$ + phenol + 4-Aminoantipyrine $\longrightarrow$ quinoneimine + $4H_2O$

Procedure

The blank, standard, and sample test tubes were filled with exactly 10 µL each of distilled water, standard solution, and serum samples respectively. After that, each test tube received 0.1 mL of cholesterol Reagent (which contains pipes buffer, 4-aminoantipyrine, phenol, peroxidase, and cholesterol esterase). Each tube's contents were mixed together and incubated for 10 minutes at 25^{o}C. Each sample's absorbance was measured at 500 nm against a blank. The procedure was performed in triplicate. The total cholesterol concentration in the serum was then calculated using the formula below:

$$\text{Cholesterol concentration (mg/dl)} = \frac{\text{Absorbance of sample x conc of standard}}{\text{Absorbance of standard}}$$

3.4.1.3 Determination of Triglycerides

The method of Tietz *et al*. (1990) was used to evaluate for triglycerides.

Principle

Lipase catalyzes the conversion of triglycerides to glycerol and free fatty acid. In the presence of ATP, the enzyme glycerol kinase reacts with the formed glycerol to produce glycerol 3 phosphate. Glycerol-3-phosphate is then oxidized to dihydroxyacetone phosphate and hydrogen peroxide as a byproduct. The oxidation of glycerol-3-phospate produces hydrogen peroxide, which is necessary for the production of red quinoneimine dye, whose intensity is proportional to the amount of triglycerides in the sample.

Triglycerides + H_2O $\longrightarrow$ glycerol + fatty acids

Glycerol + ATP $\longrightarrow$ glycerol- 3-phosphate + ADP

Glycerol- 3-phosphate + O_2 $\longrightarrow$ dihydroxyacetone-phosphate + H_2O_2

$2H_2O_2$ + 4- aminophenazone + 4 chlorophenol $\longrightarrow$ quinoneimine + HCL + $4H_2O$

Procedure

Exactly 10 µL of triglyceride standard and sample were placed into separate test tubes. After that 1 ml of reagent 1 (enzyme reagent containing 4-aminophenazone, ATP, lipases, glycerol kinase, glycerol-3-phosphate oxidase and peroxidase) was added to all the test tubes. Each test tube contents were properly mixed and incubated at 37^{o}C for 5 minutes. At 500 nm, the absorbance of the standard and the sample were measured against a reagent blank (containing only reagent 1). The test was carried out in triplicate. The triacylglycerol concentration in the serum was then calculated using the formula below:

$$\text{Triacylglycerol concentration (mg/dl)} = \frac{\text{Absorbance of sample x concentration of standard(mg/dl)}}{\text{Absorbance of standard}}$$

3.4.2 Liver Function Tests

3.4.2.1 Determination of Alanine Aminotransferase (ALT)

The method of Reitman and Frankel (1957) was used in the determination of ALT activity.

Principle

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

The sum of pyruvate hydrazone formed by 2,4-dinitrophenylhydrazine is used to determine the level of alanine aminotransferase.

Procedure

In the test tube, 100 µL of each sample was added to 500 µL of ALT R1 (alanine transaminase reagent 1 solution containing phosphate buffer, L-alanine, and - oxoglutarate). Aliquot of 0.1 mL distilled water was added to 500 µL ALT R1 solution for the blank. At 37°C, the mixtures were incubated for 30 minutes. The mixture was left to stand for 20 minutes at 25°C. At the end, aliquot of 5.0 mL of sodium hydroxide was added to all the test tubes and left to stand for 5 minutes. The sample absorbance was then compared to a reagent blank at 546 nm. The test was done in triplicate. The ALT enzyme activity in the serum was then obtained using the table in appendix ii.

3.4.2.2 Determination of Aspartate Aminotransferase (AST)

The method of Reitman and Frankel (1957) was used in the determination of AST.

Principle

α- oxoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ L-glutamate + Oxaloacetate

The amount of oxaloacetate hydrazone formed by 2,4-dinitrophenylhydrazine is used to determine aspartate aminotransferase.

Procedure

In the test tube, 100 µL of the sample was mixed with 500 µL of AST R1 solution (phosphate buffer, L-aspartate, and - oxoglutarate). The blank was made up of 100 µL distilled water and 500 µL AST R1 solution. The mixtures were incubated at 37°C for 30 minutes. Following the incubation period, all test tubes were given 500 µL of AST solution R2 (containing 2,4-dinitrophenylhydrazine). At a temperature of 20°C, the mixture was left to stand for 20 minutes. Finally, to stop the reaction, 500 µL sodium hydroxide was applied to each test tube and allowed to sit for 5 minutes. After which at the absorbance was measured at 546 nm. The test was carried out in triplicates. The AST enzyme activity in serum was then obtain using the table in appendix ii.

3.4.2.3 Determination of Alkaline Phosphatase (ALP)

The method developed by Tietz, (1976) was used in the evaluation of serum Alkaline phosphate.

Principle

$$\text{p-nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} \text{phosphate} + \text{p-nitrophenol}$$

Procedure

Exactly 500 µL of Alkaline phosphatase substrate (p-nitrophenylphosphate) was dispensed into test tubes labelled blank, standard and sample and incubated for three minutes at 37°C. Aliquot of 50 µL of distilled water, standard reagent and sample was introduced into the blank, standard and sample test tubes respectively and then mixed thoroughly. The mixture was incubated at 37°C for another 10 minutes. Exactly 2500 µL of alkaline phosphatase color developer was placed into all test tubes and mixed. At 590 nm, The Absorbance of samples were read against blank. The test was carried out in triplicates. The enzyme activity of ALP in the serum was then calculated using the formula:

$$\text{Enzyme activity of ALP (U/L)} = \frac{\text{Absorbance of sample x concentration of standard}}{\text{Absorbance of standard}}$$

3.4.2.4 Determination of Albumin

The method of Doumas *et al.* (1971) was used in the determination of the protein Albumin.

Principle

The evaluation of albumin is carried out on the basis of its quantitative binding to "3, 3', 5, 5'- tetrabromo-m cresol sulphonephthalein", which is also known as "bromocresol green (BCG)". At 578 nm, the BCG-albumin complex absorbs at a maximum capacity and the amount of albumin present is proportional to the absorbance read.

Procedure

Exactly 500 µL of distilled water was added to 3000 µL of bromocresol green (BCG) reagent in a test tube, which is the blank solution. Aliquot of 10 µL of the albumin standard was added to 3000 µL BCG reagent which served as the standard. Exactly 10 µL of the serum from each sample was also added to 3000 µL of BCG reagent. The mixtures were incubated for 5 min at 25°C. At 630 nm, the sample (A_{sample}) absorbance and also that of the standard ($A_{standard}$) were estimated against the reagent blank. The test was carried out in triplicates. The amount of albumin in the serum was then calculated using the formula below:

$$\text{Albumin (g/l)} = \frac{\text{Absorbance of sample x concentration of standard}}{\text{Absorbance of standard}}$$

3.4.2.5. Determination of Bilirubin

The method of Jendrassik and Groff (1938) was used in estimating bilirubin concentration.

Principle

In an alkaline medium, conjugated bilirubin, also known as direct bilirubin, forms a blue-colored complex with diazotized sulphanilic acid.

Procedure

Total Bilirubin

Exactly 200 µL of reagent 1 (containing sulphanilic acid and hydrochloric acid), 1000 µL of reagent 3 (containing caffeine and sodium benzoate) and 0.2 mL of the sample were mixed (served as sample blank). Exactly 0.2 mL of reagent 1, 0.05 mL of reagent 2 (containing sodium nitrite), 1mL of reagent 3 and 0.2 mL of the sample were mixed. The solutions were incubated for 10 minutes at 25°C. Aliquot of 1 mL of reagent 4 were placed after the incubation period to the solutions, thoroughly mixed and left to stand at 25°C for 5-30 min. The absorbance of the sample is read at 578 nm against the sample blank. The test was done in triplicates. The amount of total bilirubin in the serum was then calculated using the formula below:

Total Bilirubin (mg/dl) = 10.8 x A sample

3.4.2.6 Determination of Total Protein

The method by Tietz (1995) was used.

Principle

In an alkaline solution, the cupric ion interacts with protein peptide bonds, forming a coloured complex.

Procedure

Exactly 200 µL of distilled water was mixed with 1000 µL R1 (biuret reagent containing sodium hydroxide, Na-K tartrate, potassium iodide and cupric sulphate) to give the blank solution. Aliquot of 0.02 mL of standard (containing protein and sodium azide) was mixed with 1.0 mL R1 to give the standard solution, while 20 µL of the sample was mixed with 1000 µL of R1. The solutions were properly mixed and incubated at 20-25°C for 30 minutes. The sample (A_{sample}) absorbance and that of the standard ($A_{standard}$) were read against blank at 546 nm. The test was carried out in triplicates. The amount of total protein in the serum was then calculated with the formula below:

$$\text{Total Protein concentration (g/l)} = \frac{Asample}{Astandard} \text{ x Standard concentration}$$

3.4.3 Kidney Function Tests

3.4.3.1 Determination of Creatinine

The procedure defined by Bartels and Bohmer (1972) was used to evaluate creatinine concentration.

Principle

In an alkaline solution, creatinine reacts with picric acid to form a coloured complex. Creatinine accumulation determines the intensity of the coloured matrix that forms.

Procedure

The standard solution was made by mixing 1.0 mL of the working reagent (containing equal volumes of picric acid and sodium hydroxide) with 0.1 mL of the creatinine (labelled as standard), while 1.0 mL of the working reagent was mixed with 0.1 mL of the sample. The absorbance was measured at 492 nm after 30 seconds and read again after 2 minutes. The test was done in triplicate. The amount of creatinine in the serum was then calculated using the formula below:

$$\text{Creatinine concentration (mg/dl)} = \frac{\Delta Asample}{\Delta Astandard} \times 2$$

ΔA_{sample} or $\Delta A_{standard} = A_2 - A_1$

Where A_1 absorbance taken after 30 seconds,

A_2 is absorbance taken after 2 minutes.

ΔA is change in absorbance.

3.4.3.2 Determination of Urea

The system defined by Fawcett and Scott (1960) was used to estimate urea concentration.

Principle

Urease catalyses the hydrolysis of urea in the serum to produce ammonia. The ammonia concentration is then photometrically measured using Berthelot's reaction, which is a reaction between Berthelot's reagent (phenol and hypochlorite) and ammonia to yield a blue coloured compound known as indophenol.

Urea + $H_2O \longrightarrow 2NH_3 + CO_2$

NH_3 + Hypochlorite + Phenol $\longrightarrow$ Indophenol (Blue compound)

Procedure

Blank solution for this test was obtained by mixing exactly 0.01 mL of distilled water with 0.1 mL of reagent 1 (EDTA, sodium nitropusside and urease). Exactly 0.01 mL of the standard was thoroughly mixed with 0.1 mL of reagent 1 (standard solution), while

0.01 mL of the sample was also thoroughly mixed with 0.1 mL of reagent 1. The contents were incubated at 37°C for 10 minutes. Aliquot of 2.5 mL of reagent 2 (containing phenol) and 2.5 mL of reagent 3 (containing hypochlorite) were added to the test tubes, thoroughly mixed and incubated for 15 minutes at 37°C. The sample (A_{sample}) absorbance and that of the standard ($A_{standard}$) were read against blank at 546nm. The test was carried out in triplicate. The amount of urea in the serum was then calculated using the formula below:

$$\text{Urea concentration (mg/dl)} = \frac{Asample}{Astandard} \times \text{Standard concentration}$$

3.4.4 Blood Electrolyte Test

Procedure

Exactly 15 µl of the plasma sample was aspirated by Ion Selective Electrode (ISE). The result of Na^+, K^+, HCO_3^- and Cl^- were displayed on the screen of the automated electrolyte analyser in mMol/L.

3.5 Haematological Parameters

A 5ml syringe was used to extract blood samples for haematological analysis into an EDTA bag. A multi-parameter, automatic haematology analyser, the URIT-3010, was used to conduct the study. Red Blood Cell Count, White Blood Cell Count, Mean Corpuscular Haemoglobin Concentration, Mean Corpuscular Haemoglobin, Packed Cell Volume, Platelet Count, Monocyte Count, Lymphocyte Count, Mean Corpuscular Volume, Granulocyte Count, Red Blood Cell Distribution Width, and Haemoglobin Concentration are among the blood parameters that are measured.

3.6 Histopathology

The following organs kidney, liver, colon, testes and heart were harvested, weighed and preserved in 10 % formalin. The organs were then removed, and tissue portions with a thickness of 5 μm were stained with haematoxylin and eosin (H&E) for histopathological study. The stained sections were subsequently mounted on a microscope, viewed and their photomicrographs taken (Magnification x100). The relative organ weight of the kidney, heart, and liver were calculated as organ/body weight x 100 % (Hor *et al.*, 2011).

3.7 Sex Hormone

A 5ml syringe was used to retrieve the blood samples, which were then placed in an EDTA tube. The following approaches were used to assess follicle stimulating hormone (FSH), luteinizing hormones (LH), estradiol, prolactin, progesterone, and testosterone.

3.7.1 Follicle Stimulating Hormone (FSH)

This was evaluated using the FSH ELISA kit

Principle

The FSH ELISA package is a solid phase assay with immobilized streptavidin on each well. The samples are placed in the streptavidin-coated wells, along with the anti-FSH/anti-Biotin conjugate. FSH is sandwiched between biotin-labeled antibodies and HRP in the patient's plasma. Wash buffer is used to remove unbound proteins and HRP conjugates. The colour intensity is proportional to the concentration of FSH in the samples when the substrate is added.

Procedure

Aliquot of 0.05 mL of FSH standards, control and sample were pipetted into their designated wells, after which 0.1 mL of enzyme conjugates were then added to all wells, the well covered and incubated for 60 minutes at room temperature. At the end of the incubation period, the contents from each well were removed and wells washed thrice with 0.3 mL of wash solution. Absorbent paper towels were used to blot the well to remove excess solution. Aliquot of 100 μL of "3,3',5,5' tetramethylbenzidine (TMB) substrate" was then added to the wells and incubated at room temperature for 15 minutes. Exactly 50 µL of the stop solution was added to the wells at the end of incubation period. The microplate wells were gently shaken and absorbance read at 450 nm using an ELISA reader within 15 minutes after adding the stop solution.

3.7.2 Testosterone

This was evaluated using the Testosterone ELISA kit

Principle

The Testosterone ELISA kit is a competitive solid phase ELISA. The tests, along with a working testosterone-horseradish peroxidase (HRP) conjugate and an anti-Testosterone-Biotin solution, are placed in streptavidin-coated wells. Testosterone in the plasma competes for binding sites with the testosterone-HRP conjugate Washing buffer is used to remove unbound Testosterone and Testosterone enzyme conjugate. The intensity of the colour is inversely proportional to the concentration of Testosterone in the samples after the substrate is added.

Procedure

Aliquot of 0.05 mL of the standards, control and samples were pipetted into the designated wells, after which 0.1 mL of working testosterone -enzyme conjugate reagent were then added to all wells and immediately followed by 0.05 mL biotin reagent. The microplate wells were then swirled for 20-30 seconds so the reagent can mix properly, after which they were covered and incubated for 60 minutes at room temperature. The liquid was removed from all wells after the incubation period and then washed three times with 300 µL of 1X wash buffer. The wells were blotted on absorbent paper towels to remove any of the remain solution. Exactly 100 µL of 3,3',5,5'- tetramethylbenzidine (TMB) substrate was then added to all wells and incubated at room temperature for 30 minutes. Aliquot of 50 µL of stop solution was added to all well at the end of the incubation period. The wells were gently swirled for 15-20 seconds to mix the solution and absorbance read on an ELISA reader at 450 nm within 15 minutes after adding the stop solution.

3.7.3 Luteinizing Hormone

The LH ELISA kit was used to assess this.

Principle

The LH ELISA package is a solid-phase assay that uses streptavidin and biotin to detect LH. The interaction of the streptavidin coated well and the exogenously bound biotinylated monoclonal anti-LH antibody causes immobilization at the surface of a microplate well during the assay Without competition or steric hindrance, the native antigen and antibodies react to form a soluble sandwich complex. The complex is simultaneously stored in the well using a high affinity reaction with streptavidin and biotinylated antibody. After a one-hour incubation cycle, unbound protein and conjugate are washed away with wash buffer. After adding the substrate, the enzyme activity in the enzyme bound fraction is proportional to the concentration of LH in the samples.

Procedure

Aliquot of 25 µL of control, FSH standards, and samples were placed into selected wells, after which 100 µL of 1X biotin/enzyme conjugates were added to all wells. The microplate wells were covered and allowed to stand at room temperature for 60 minutes. At the end of the one-hour period, the wells were emptied of their liquid content and washed thrice using 300 µL of 1X wash buffer. Absorbent paper towels were then used to blot out excess liquid from the wells. Aliquot of 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate was then added to all wells and left to stand for 15 minutes at room temperature. At the conclusion of the 15-minute incubation period, 50 µL of stop solution was applied to each well. Within 15 minutes after applying the stop solution, it was gently shaken to mix the solution, and absorbance was measured at 450 nm on an ELISA reader.

3.7.4 Estradiol Hormone

This was determined using the estradiol E2 ELISA kit

Principle

The estradiol E2 ELISA package works on the basis of competitive binding between E2 in the test specimen and E2 enzyme conjugate in exchange for a constant volume of anti-estradiol polyclonal antibody. During the incubation, anti-E2 antibody coated wells are incubated at room temperature with E2 standards, control, tests sample, and E2 enzyme conjugate for 60 minutes. During the incubation, a fixed volume of HRP-labelled E2 competes with the endogenous E2 in the normal, sample, for a fixed number of binding sites of the individual E2 antibody. If the concentration of E2 in the specimen rises, the immunologically bound E2 peroxidase conjugate in the well decreases. The wells are then washed to eliminate any unbound E2 peroxidase conjugate. After that, a "TMB reagent solution" is applied and incubated for 30 minutes at room temperature, resulting in the formation of blue colour. With the addition of stop solution, the colour production is halted, and the absorbance is determined spectrophotometrically at 450 nm.

Procedure

Coated strip microplate wells were placed into the holder. Aliquot of 25 µL of standards, control and samples were pipetted into assigned wells, after which 50 µL of working solution of estradiol biotin reagent were then added to all wells. The wells were mix for 10-20 seconds by placing on shaker. The wells were then covered and incubated for 45minutes at room temperature. After the incubation period, 100 µL of estradiol enzyme reagent were added to all wells, mix for 10-20 seconds by placing on shaker and was incubated for another 45 minutes. After the second incubation, the liquid was removed from all wells and washed three times with 300 µL of 1X wash buffer. The wells were then blotted on absorbent paper towels. Exactly 100 µL of TMB substrate was then added to all wells and incubated for another 20minutes at room temperature. At the end of the 20-minute incubation period, exactly 50 µL of stop solution was then added to all wells. The wells were swirled gently to allow the solution mix properly and absorbance read on an ELISA reader at 450 nm within 15 minutes after adding the stop solution.

3.7.5 Progesterone

This was determined using the progesterone ELISA kit

Principle

The progesterone ELISA kit is a solid phase competitive ELISA. The samples, working progesterone-enzyme (HRP) conjugate and anti- progesterone -Biotin reagent are added to the wells coated with streptavidin. Progesterone in the sample competes with the progesterone HRP conjugate for binding sites. Unbound progesterone and progesterone enzyme conjugate is washed off by washing buffer. Upon the addition of the TMB substrate, colour develops. The intensity of the colour is inversely proportional to the concentration of progesterone in the samples. A standard curve is prepared relating colour intensity to the concentration of the progesterone.

Procedure

Coated strip wells were placed into the holder. Aliquot of 20µL of the progesterone standards, control and samples were pipetted into selected wells, after which 100 µL of working progesterone -enzyme conjugates were then added to all wells, followed by 50 µL progesterone biotin conjugate. The wells were then covered and incubated at room temperature for 60 minutes, after the incubation period, the liquid was removed from all wells and the wells washed three times with 300 µL of 1X wash buffer. The wells were then blotted on absorbent paper towels. Exactly 100 µL of TMB substrate was then added to all wells and incubated at room temperature for 15 minutes. Aliquot of 50 µL of stop solution was added to all well at the end of the 15-minute incubation time. The wells were swirled gently to mix the solution and absorbance was read on an ELISA reader at 450 nm within 15 minutes after adding the stop solution.

3.7.6 Prolactin

This was determined using the Calbiotech prolactin ELISA kit

Principle

The prolactin ELISA kit is a solid phase sandwich ELISA assay method based on the streptavidin-biotin principle. The standards, samples, and a reagent mixture of anti-prolactin enzyme and biotin conjugate are added into the wells coated with streptavidin. Prolactin in the plasma forms a sandwich between two specific prolactin antibodies, labelled with biotin and horseradish peroxidase (HRP). Simultaneously, the biotinylated antibody is immobilized onto the well through a high affinity Streptavidin-Biotin interaction. Unbound protein and excess biotin/enzyme conjugated reagent are washed off by washing buffer. Upon the addition of the substrate, the intensity of the colour developed is directly proportional to the concentration of prolactin in the samples. A standard curve is prepared relating colour intensity to the concentration of the prolactin

Procedure

Coated strip wells were placed into the holder. Aliquot of 25 µL of the prolactin standards, control and samples were pipetted into assigned wells, after which 100 µL of enzyme conjugates were then added to all wells. The wells were then covered and incubated at room temperature for 60 minutes. The liquid was removed from all wells and washed three times with 300 µL of 1X wash buffer after the incubation period. The wells were then blotted on absorbent paper towels. Exactly 100 µL of TMB substrate was then added to all wells and incubated for 15 minutes at room temperature. Aliquot of 50 µL of stop solution was added to all well at the end of the 15 minutes' incubation time. The wells were swirled gently to allow the solution mix properly and absorbance read on an ELISA reader at 450 nm within 15 minutes after adding the stop solution.

3.8 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Gas chromatography-mass spectrophotometric analysis was carried out using methanol and aqueous extracts of the pulverized samples of both the pulp and seeds of the fruits. This was carried out to identify and quantify all bioactive compounds present in the samples. Identification and quantification were done according to the retention time and peak formation of the compounds.

Gas chromatography (Agilent technologies, 7890 GC system) and a Mass Spectrometer (MS) detector were used for the study (Agilent technologies 5975). The Agilent H5MS column was used, which had a length of 30 meters, a diameter of 0.320 millimeters, and a thickness of 0.25 millimeters. With a flow rate of 0.5 ml/min, helium gas served as the carrier gas. For the study, a one-microliter sample injection volume was used. The oven temperature was set to 80 degrees Celsius for 2 minutes, then gradually increased by 10 degrees Celsius per minute until it reached 240 degrees Celsius for 6 minutes. The entire run time lasted for 90 minutes. For compound identification and quantification, GCMS data were analyzed using electron impact ionization at 70 eV and total ion count (TIC). The spectrum of components was linked to a catalog of known compound spectrums contained in the National Institute of Standards and Technology's (NIST) data set. Turbo-mass OCPTVS-demo SPL software was used to measure peak areas and process the results.

3.9 Data Analysis

All measurements were done in triplicate and results are presented in chapter four as mean ± standard error of mean (SEM). Data were analysed using one-way analysis of variance (ANOVA) and differences between means were determined using the least significant difference on Statistical Package for Social Sciences (SPSS) version 16. The significance level was set at $p < 0.05$.

CHAPTER FOUR

RESULTS

4.1. Effect of aqueous-methanol pulp extract of *Azanza garckeana* on body weight of male Wistar albino rats

The effect of the aqueous-methanol pulp extract of *Azanza garckeana* on body weight of animal is shown in Table 4.1. There was a non-significant increase ($p > 0.05$) in body weight of test rats compared with the control.

4.2 Effect of aqueous-methanol pulp extract of *Azanza garckeana* on relative organ weights of male Wistar albino rats

The oral administration of aqueous-methanol extract of *Azanza garckeana* for 28 days did not produce any significant change ($p > 0.05$) in the relative weight of the organs (kidney, heart, and liver) compared with the animals in the control group (Table 4.2).

4.3 Effect of aqueous-methanol pulp extract of *Azanza garckeana* on biochemical parameters (lipid profile, liver and kidney function tests) in male Wistar albino rats

4.3.1. Lipid profile tests

The results showed that aqueous-methanol pulp extract of *Azanza garckeana* did not significantly alter serum total cholesterol, triacylglycerol, HDL and LDL concentrations as shown in Table 4.3 below.

4.3.2 Liver function tests

Table 4.4 show the effect of the aqueous-methanol pulp extract of *Azanza garckeana* on liver function parameters of rats. The three doses did not significantly alter liver enzymes such as ALT, AST and ALP as well as the concentrations of total protein, albumin, total bilirubin and conjugate bilirubin when compared with the control.

Table 4.1: Effect of aqueous-methanol pulp extract of *Azanza garckeana* on mean body-weight of male Wistar rats

Group	Mean Initial Body Weight (g)	Mean Final Body Weight (g)	Change in Body weight(g)
Control	205.67 ± 6.33	244.33 ± 2.96	38.67 ± 6.36
50mg/kg B.Wt	210.33 ± 3.71	255.33 ± 8.67	45.00 ± 6.11
300mg/kg B.Wt	194.33 ± 9.84	242.67 ± 14.17	48.33 ± 4.37
2000mg/kg B.Wt	211.67 ± 2.85	247.00 ± 1.00	35.33 ± 1.86

Data reported as mean ± standard error of mean, n=3. Values with superscript [a] are significantly different ($p \leq 0.05$) from the control group.

Table 4.2: Effect of aqueous-methanol pulp extract of *Azanza garckeana* on relative organ-weight (%) in male Wistar rats

Group	Kidney (%)	Heart (%)	Liver (%)
Control	0.65 ± 0.06	0.33 ± 0.02	3.14 ± 0.31
50mg/kg B.Wt	0.62 ± 0.03	0.37 ± 0.03	3.06 ± 0.23
300mg/kg B.Wt	0.61 ± 0.06	0.27 ± 0.02	3.31 ± 0.21
2000mg/kg B.Wt	0.57 ± 0.02	0.34 ± 0.01	2.85 ± 0.01

Data reported as mean ± standard error of mean, n=3. Values with superscript [a] are significantly different ($p \leq 0.05$) from the control group.

Table 4.3: Effect of aqueous-methanol pulp extract of *Azanza garckeana* on lipid profile in male Wistar albino rats

Group	TAG (mg/dl)	T. CHOL (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Control	95.93 ± 24.37	65.07 ± 7.64	15.77 ± 1.23	30.17 ± 9.17
50 mg/kg B.Wt	100.00 ± 6.92	99.17 ± 34.92	30.30 ± 13.85	48.87 ± 20.19
300 mg/kg B.Wt	92.67 ± 14.77	84.87 ± 19.45	25.43 ± 15.61	40.87 ± 7.52
2000 mg/kg B.Wt	81.23 ± 25.08	80.93 ± 14.35	16.13 ± 2.87	48.57 ± 8.08

Data reported as mean ± standard error of mean, n=3. Values with superscript [a] are significantly different ($p \leq 0.05$) from the control group.

Legend

TAG: Triacylglycerol

T. CHOL.: Total Cholesterol

HDL-C: High density lipoprotein- Cholesterol

LDL-C: Low density lipoprotein- Cholesterol

Table 4.4: Effect of aqueous-methanol pulp extract of *Azanzagarckeana* on liver function tests in male Wistarrats

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	T. PROT (g/l)	T. BIL (mg/dl)	Albumin (g/l)	C. BIL (mg/dl)
Control	24.57 ± 6.25	9.27 ± 1.78	99.30 ± 3.52	71.43± 1.99	1.03± 0.38	34.59± 5.11	0.31 ± 0.09
50 mg/kg B.Wt	36.49 ± 13.89	12.00 ± 1.44	97.64 ± 4.18	75.68±9.56	0.57± 0.16	50.13± 7.08	0.34 ± 0.08
300 mg/kg B.Wt	37.59 ± 6.05	11.17 ± 0.41	117.80 ± 10.66	68.21± 6.96	0.74± 0.03	42.89± 1.19	0.45 ± 0.06
2000 mg/kg B.Wt	38.08 ± 5.58	7.33 ± 1.19	105.10 ± 9.30	74.73± 10.48	0.73± 0.17	42.53± 3.97	0.47 ± 0.12

Data reported as mean ± standard error of mean (SEM), n=3. Values with superscript [a] are significantly different ($p \leq 0.05$) from the control group.

Legend

ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
ALP: Alkaline phosphatase
T. PROT.: Total protein
T. BIL: Total bilirubin
C. BIL.: Conjugated bilirubin

4.3.3 Kidney function tests

Table 4.5 shows the effect of aqueous-methanol pulp extract of *Azanza garckeana* on kidney function tests of male Wistar albino rats. The creatinine levels increased in the three test groups (50, 300 and 200mg/kg) but only those administered 300 and 2000mg/kg body weight of the extract were found to be significantly different ($p < 0.05$) compared to the control group. However, a non-significant effect ($p > 0.05$) in urea levels was observed compared with control. There was no significant difference ($p > 0.05$) in the values of sodium ion, potassium ion, chloride ion and bicarbonate ion concentrations between the test groups relative to the control group.

4.4 Effect of aqueous-methanol pulp extract of *Azanza garckeana* on haematological parameters in male Wistar albino rats

The effect of the aqueous-methanol pulp extract of *Azanza garckeana* on haematological parameters in male Wistar albino rats is shown in Table 4.6.

The result shows that aqueous-methanol pulp extract of *Azanza garckeana* has no significant difference ($p > 0.05$) on white blood cell, lymphocyte, monocyte and granulocyte count in rats as shown in Table 4.6. The result also shows a non-significant change ($p > 0.05$) in RBC, HGB and MCHC of rats administered aqueous-methanol pulp extract of *Azanza garckeana* at doses of 50, 300 and 2000 mg/kg body weight compared with those of control. However, a significant decrease ($p < 0.05$) in RDW was observed for the three test groups administered 50,300 and 2000mg/kg body weight of the extract relative to control group.

More so, the result of this study also showed a non-significant effect ($p > 0.05$) on Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) in the male Wistar albino rats (Table 4.6). There was a slight decrease in platelet distribution width (PDW) and mean platelet volume (MPV)

Table 4.5: Effect of aqueous-methanol pulp extract of *Azanzagarckeana* on kidney function tests in male Wistarrats

Group	Creat. (mg/dl)	Urea (mg/dl)	Sodium (mmol/l)	Potassium (mmol/l)	Chloride (mmol/l)	Bicarb. (mmol/l)
Control	0.71± 0.09	34.16 ± 0.48	136.77 ± 2.46	7.68 ± 0.57	107.80 ±0.80	14.20 ± 0.95
50 mg/kg B.Wt	0.78 ± 0.03	27.40 ± 2.74	139.00 ± 1.79	7.95 ± 0.28	107.90 ±0.98	15.87 ± 1.93
300 mg/kg B.Wt	1.08 ± 0.69[a]	35.31 ± 1.25	138.77 ± 0.93	8.45 ± 0.19	108.07 ± 0.44	13.17 ± 2.95
2000 mg/kg B.Wt	1.01 ± 0.16[a]	29.47 ± 3.71	138.20 ± 0.99	8.56 ± 0.73	110.33 ± 2.53	15.83 ± 1.57

Data reported as mean ± standard error of mean (SEM), n=3. Values with superscript [a] are significantly different ($p \leq 0.05$) from the control group.

Legend

Creat.: Creatinine
Bicarb.: Bicarbonate

Table 4.6: Effect of aqueous-methanol pulp extract of *Azanzagarckeana* on haematology in male Wistarrats

Group	WBC (10^3 cells/ µL)	LYM (10^3 cells/ µL)	MON (10^3 cells/ µL)	GRAN (10^3 cells/ µL)	LYM (%)	MON (%)	GRAN (%)	RBC (10^{12} cells /L)	HGB (g/L)	MCHC (g/L)	RDW (%)	MCV (fL/cell)	MCH (pg/cell)	PLT (x 10^9 cells/L)	PCT (%)	PDW (%)	MPV (fL)
Control	5.67± 2.10	4.37± 1.63	0.37± 0.13	0.93± 0.38	76.9± 2.29	5.93± 0.73	17.17± 2.09	5.81± 1.61	14.8± 4.66	33.30± 0.00	16.2± 0.70	74.69± 6.41	24.90± 2.15	4.16± 1.25	0.4± 0.15	9.70± 1.29	9.63± 0.87
50 mg/kg B.Wt	2.27± 0.62	1.60± 0.40	0.16± 0.07	0.50± 0.15	71.53± 1.39	6.63± 0.43	21.83± 1.09	3.83 ± 0.66	10.3± 1.93	33.27± 0.03	12.9± 0.46[a]	80.92± 2.22	26.93± 0.73	0.64± 0.27[a]	0.05 ± 0.03[a]	8.20 ± 0.00	8.00 ± 0.61
300 mg/kg B.Wt	3.73± 1.15	3.07± 0.84	0.23± 0.09	0.43± 0.24	82.73± 3.17	5.67± 0.54	11.60±2.96	4.32± 0.71	11.1± 1.99	33.33± 0.06	14.3± 0.45[a]	76.88± 1.30	45.60± 19.65	1.2± 0.20[a]	0.1± 0.02[a]	8.80± 0.60	8.93± 0.58
2000 mg/kg B.Wt	4.63± 2.13	3.23± 1.92	0.30± 0.20	1.10 ± 0.32	0.73± 0.17	42.53± 3.97	0.47 ± 0.12	7.16 ± 0.52	16.9± 0.40	33.30± 0.00	13.3± 0.47[a]	75.97± 3.88	23.70± 1.30	0.69± 0.45[a]	0.7± 0.03[a]	8.20± 0.00	7.97± 0.50

Data reported as mean ± standard error of mean (SEM), n=3. Values with superscript [a] are significantly different ($p \leq 0.05$) from the control group.

Legend

WBC: White blood cell
LYM: Lymphocyte
MON: Monocyte
GRAN: Granulocyte
RBC: Red blood cell
HGB: Haemoglobin
MCHC: Mean corpuscular haemoglobin concentration
RDW: Red cell distribution width
MCV: Mean corpuscular volume
MCH: Mean corpuscular haemoglobin
PLT: Platelet
PCT: Platelet crit
PDW: Platelet distribution width
MPV: Mean platelet volume

4.5 Effect of the aqueous-methanol pulp extract of *Azanza garckeana* on the histopathological parameters of male Wistar albino rats

Histopathological examination of the liver, kidney, heart, testes and colon tissues of rats administered aqueous-methanol pulp extract of *Azanza garckeana* at doses of 50, 300 and 2000 mg/kg bodyweight revealed no alterations or adverse effects (plate 4.1 - plate 4.20).

4.5.1 Liver

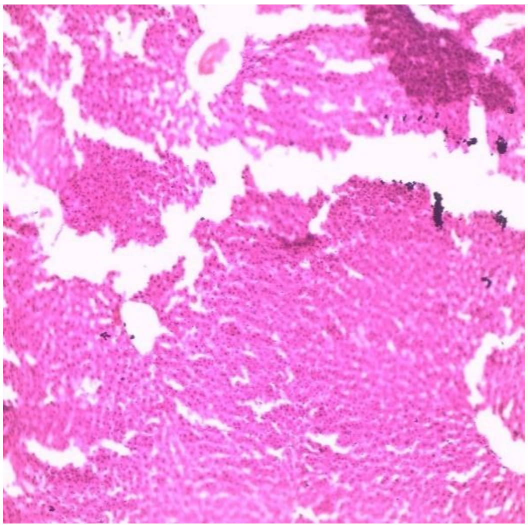


Plate 4.1: Photomicrograph of the liver of control rat (H+E x100)

Section shows normal plates of hepatocyte separated by sinusoids and normal central vein.

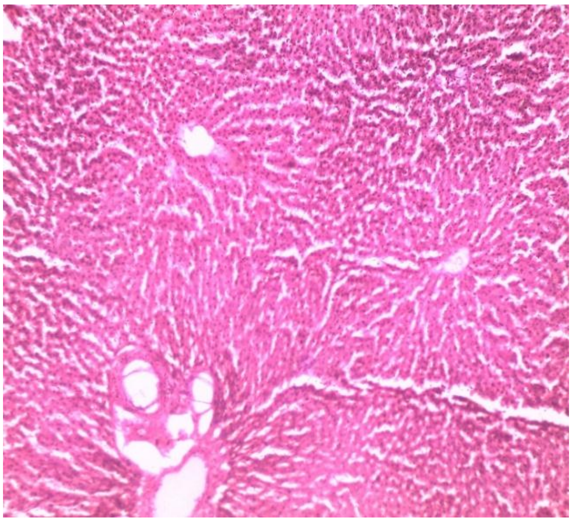


Plate 4.2: Photomicrograph of the liver of rat administered 50 mg/kg b.wt extract (H+E x100)

Section shows normal plates of hepatocyte.

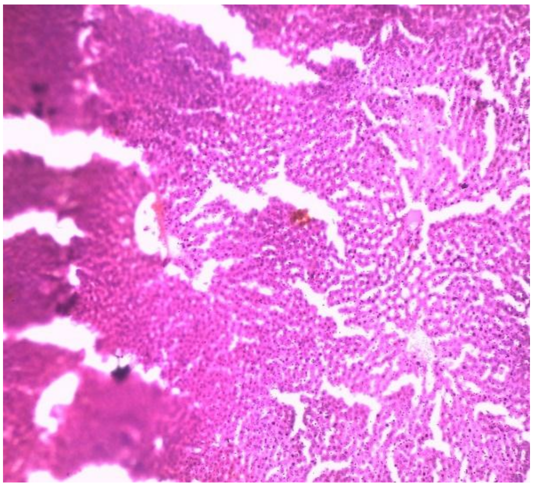


Plate 4.3: Photomicrograph of the liver of rat administered 300 mg/kg b.wt extract (H+E x100)

Section shows normal plates of hepatocyte.

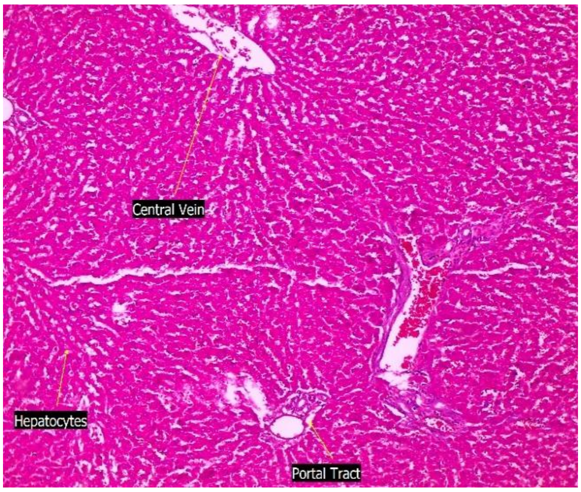


Plate 4.4: Photomicrograph of the liver of rat administered 2000 mg/kg b.wt extract (H+E x100).

Essentially normal liver sections show central vein, plates of hepatocyte separated by sinusoids.

4.5.2 Kidney

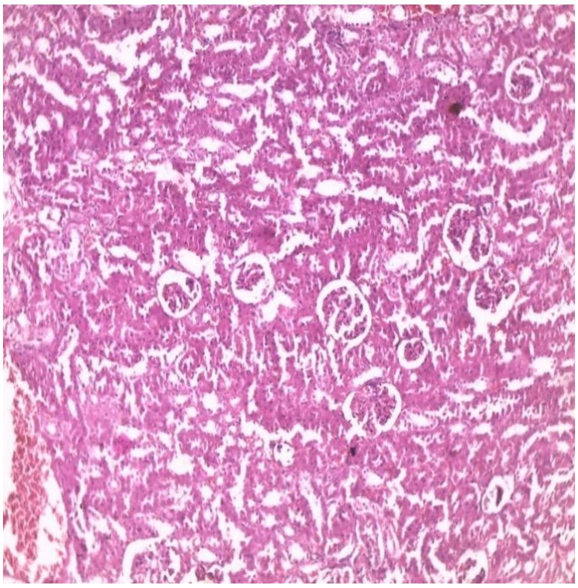


Plate 4.5: Photomicrograph of the kidney of control rats (H+E x100)

Sections show normal kidney consisting of glomeruli tubules and blood vessel

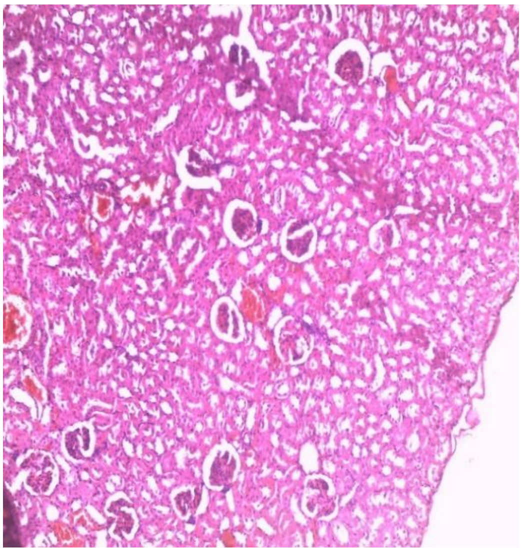


Plate 4.6: Photomicrograph of the kidney of rats administered 50 mg/kg b.wt extract (H+E x100)

Sections of the kidney showing normal glomeruli, tubules and interstitium

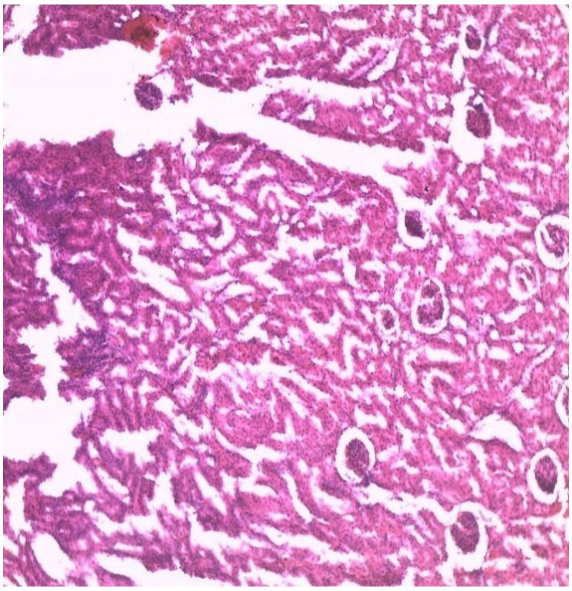


Plate 4.7: **Photomicrograph of the kidney of rats administered 300 mg/kg b.wt extract (H+E x100) .**

Sections show normal kidney consisting of glomeruli tubules and blood vessels

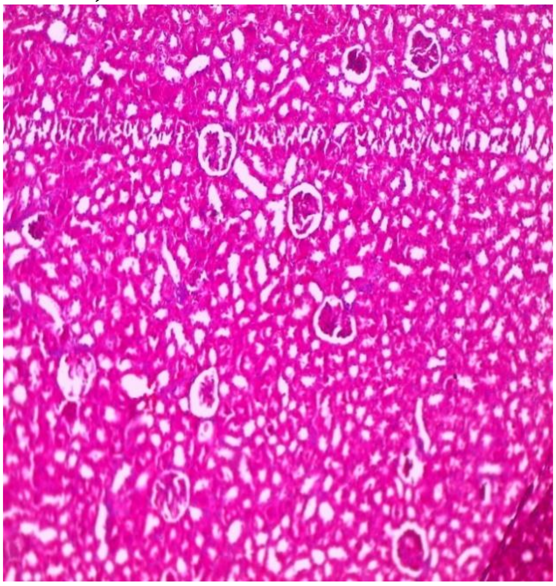


Plate 4.8: **Photomicrograph of the kidney of rats administered 2000 mg/kg b.wt extract(H+E x100)**

Sections of the kidney showing normal glomeruli, tubules and interstitium

4.5.3 Heart

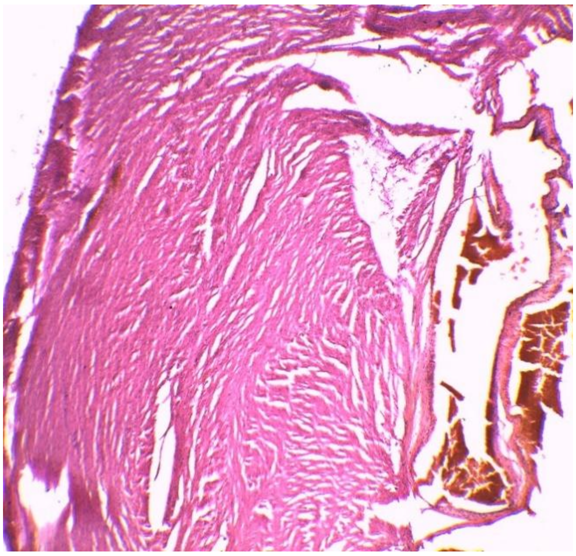


Plate 4.9: Photomicrograph of the heart of control rat (H+E x100)
Heart architecture showing normal ıyocardium.

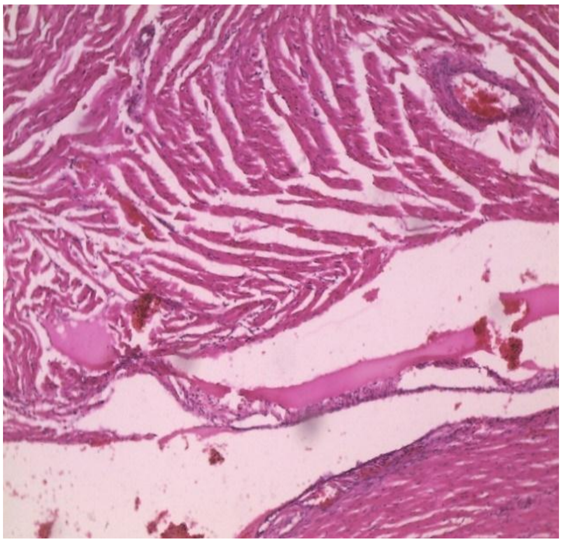


Plate 4.10: Photomicrograph of the heart of rat administered 50 mg/kg b.wt extract (H+E x100)
Heart architecture showing normal myocardium.

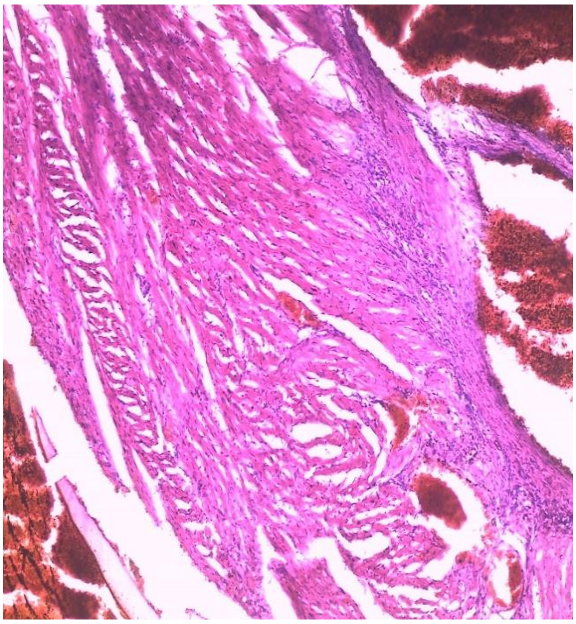


Plate 4.11: Photomicrograph of the heart of rat administered 300 mg/kg b.wt extract (H+E x100)
Heart architecture showing normal myocardium.

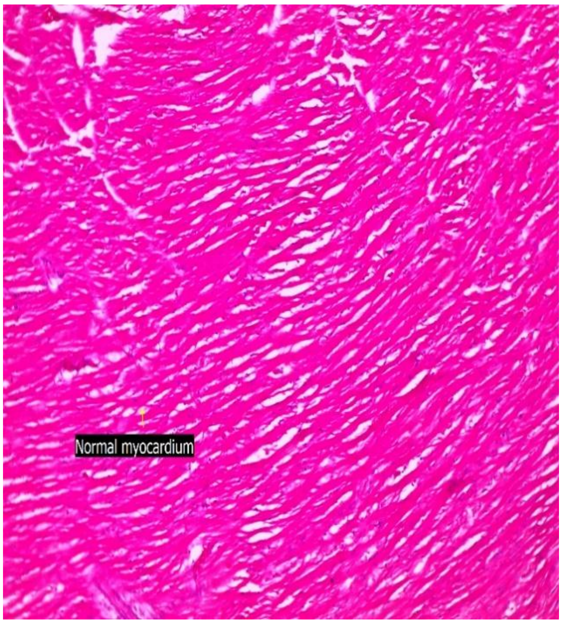


Plate 4.12: Photomicrograph of the heart of rat administered 2000 mg/kg b.wt extract (H+E x100).
Heart architecture showing normal myocardium.

4.5.4 Testes

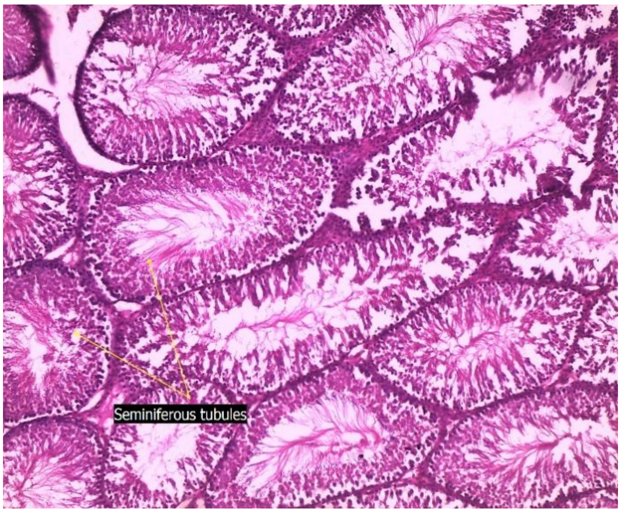


Plate 4.13: Photomicrograph of the testes of control rat (H+E x100)
Histologic section showing normal seminiferous tubules that are lined by cells of the spermatogenic series and separated by interstitium

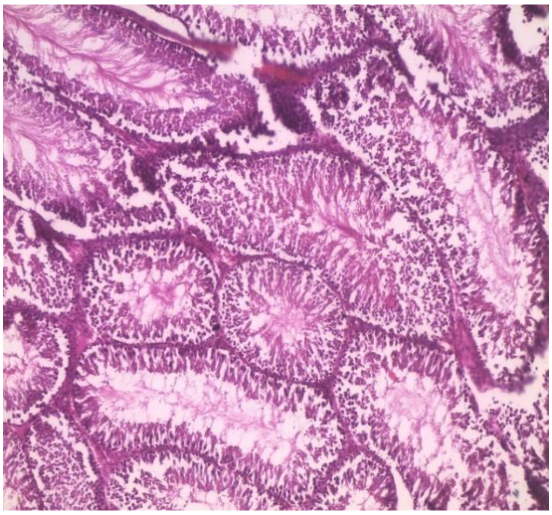


Plate 4.14: Photomicrograph of the teste of rat administered 50 mg/kg b.wt extract (H+E x100)
Histologic section showing normal seminiferous tubules that are lined by cells of the spermatogenic series.

Plate 4.15: Photomicrograph of the testes of rat administered 300 mg/kg b.wt extract (H+E x100)
Histologic section showing normal seminiferous tubules that are lined by cells of the spermatogenic series.

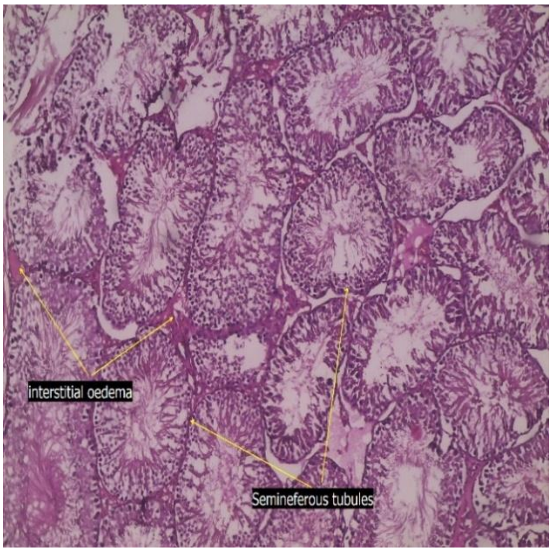


Plate 4.16: Photomicrograph of the testes of rat administered 2000 mg/kg b.wt extract (H+E x100).
Histologic section showing normal seminiferous tubules that are lined by cells of the spermatogenic series and separated by interstitial. There is interstitial oedema.

4.5.5 Colon

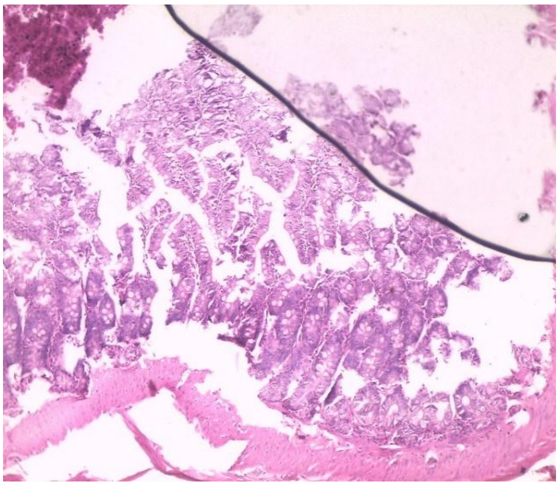


Plate 4.17: Photomicrograph of the colon of control rat (H+E x100)

Section shows colon with normal mucosa containing goblet cells and laminapropria

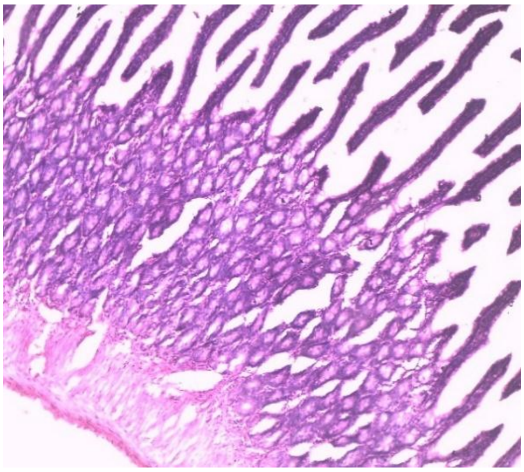


Plate 4.18: Photomicrograph of the colon of rat administered 50 mg/kg b.wt extract (H+E x100)

Section shows colon with normal mucosa containing goblet cells and laminapropria

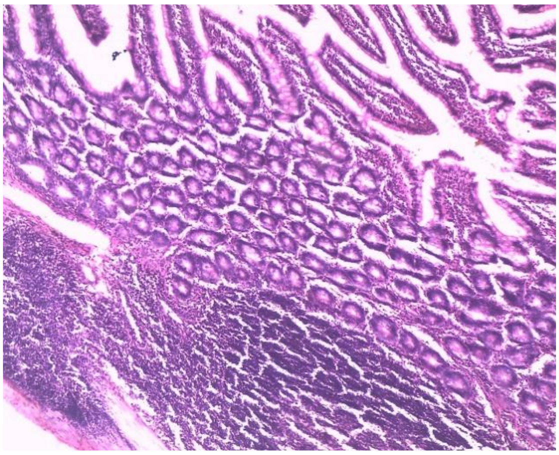


Plate 4.19: Photomicrograph of the colon of rat administered 300 mg/kg b.wt extract (H+E x100)

Section shows colon with normal mucosa glands lined by columnar epithelium and goblet cells.

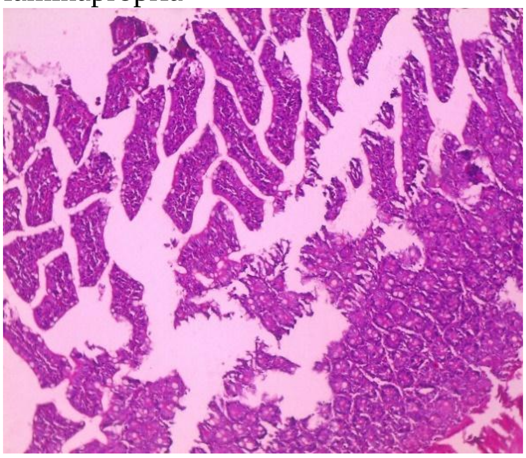


Plate 4.20: Photomicrograph of the colon of rat administered 2000 mg/kg b.wt extract (H+E x100).

Sections showing mucosal epithelium with goblet cells

4.6 Effect of the aqueous-methanol pulp extract of *Azanza garckeana* on sex hormones of male Wistar albino rats

The effect of oral administration of aqueous–methanol pulp extract of *Azanza garckeana* on sex hormones levels of male Wistar albino rats (Table 4.7) showed a slight decrease in progesterone level in rats administered 50 mg/kg body weight of the extract, which was not significantly different when compared to control; whereas the progesterone levels of rats administered 300 and 2000 mg/kg body weight of the extract were significantly elevated ($p < 0.05$) compared with the control. There was a non-significant decrease ($p > 0.05$) in LH and prolactin levels in the test group administered 50 and 300 mg/kg body weight, while those of the 2000 mg/kg body weight test group increased but were not significantly different ($p > 0.05$) compared with control. There was a non-significant decrease ($p > 0.05$) in testosterone, estradiol and FSH levels in the three test groups administered 50, 300 and 2000 mg/kg body weight of the extract when compared with control.

4.7 GC-MS analysis of *Azanza garckeana* fruit (Pulp and Seed)

4.7.1 GC-MS result from the analysis of the methanol pulp extract of *Azanza garckeana*

The total ion chromatogram (TIC) showing the peak of the various bioactive compounds found in the methanol extract of *Azanza garckeana* pulp are shown in Figure 4.1 with a total of 23 bioactive compounds identified and quantified using their peak and retention time (RT). The compound 5-hydroxyl methyl furfural was found to be the highest in the methanol extract of the pulp of *Azanza garckeana* with 19.39 % in content and a retention time (RT) of 7.739, followed by 2R, 3S-9 (1,3,4- trihydroxyl)– 2 butoxymethylguanine (13.60 % in content and RT of 12.14), while N-methyl-3-hydroxymethylpyrrolidin was the least with 0.10 % in content and retention time of 4.173**.** The compounds found in the methanol extract of the pulp of *Azanza garckeana*,

Table 4.7: Effect of the aqueous-methanol pulp extract of *Azanza garckeana* on sex hormones levels of male Wistar albino rats

Group	LH (mIU/l)	PRO (ng/ml)	FSH (mlU/l)	PROGES (ng/ml)	TEST (ng/ml)	E2 (pg/ml)
Control	2.40±0.06	2.90 ± 0.10	4.67 ±1.07	4.87± 0.20	1.53±0.09	10.67±0.43
50mg/kg B.Wt	2.33 ±0.07	2.80 ± 0.20	3.47 ±0.15	4.80± 0.10	1.43±0.23	9.97±0.15
300mg/kg B.Wt	2.57±0.07	3.10 ± 0.35	3.90 ±0.15	9.90 ± 0.97^{a}	1.43±0.89	10.20±0.06
2000mg/kg B.Wt	2.37±0.07	2.83 ± 0.12	3.90 ± 0.06	6.93 ± 0.74^{a}	1.43±0.15	10.53±0.09

Data reported as mean ± standard error of mean n=3. Values with superscript ^{a} are significantly different ($p \leq 0.05$) from the control group.

Legend

LH: Luteinizing hormone

PRO: Prolactin

FSH: Follicle stimulating hormones

PROGES: Progesterone

TEST: Testosterone

E2: Estradiol

along with their biological activities, retention time (RT), molecular formula, molecular weight and concentration (% Area) are presented in Table 4.8 below.

4.7.2 GC-MS result from the analysis of the aqueous extract of *Azanza garckeana* pulp

Peaks of the GC-MS compounds detected in the aqueous extract of *Azanza garckeana* pulp are shown below in the total ion chromatogram (figure 4.2). A total of 18 bioactive compounds were identified and quantified using their peak and retention time. Heneicosane was found to be the highest in the aqueous extract with 9.01 % in content and RT of 14.812 min, while dodecane 2methyl, 6-propyl, was found to be the least with 1.54 % and RT of 12.756 min. The compounds found in the aqueous extract of the pulp of *Azanza garckeana*, along with their biological activities, retention time (RT), molecular formula, molecular weight and concentration (% Area) are presented in Table 4.9 below.

4.7.3 GC-MS result from the analysis of methanol extract of *Azanza garckeana* seed

The total ion chromatogram (TIC) showing the peaks of the various bioactive compounds that are found in the methanol extract of *Azanza garckeana* seeds are shown in Figure 4.3. A sum of 17 bioactive compounds were identified and quantified using their peak and retention time. The compound n-hexadecanoic acid was found to be the highest in the methanol extract of the seed of *Azanza garckeana* with a concentration of 30.44 % and a retention time (RT) of 15.09 min, followed by 9,12 Octadecadienoic acid (Z, Z) (24.32 % and RT of 16.496 min), 9-Octadecenoic acid (Z)- methyl ester was the least with a concentration of 0.33 % and retention time of 16.09 min**.**

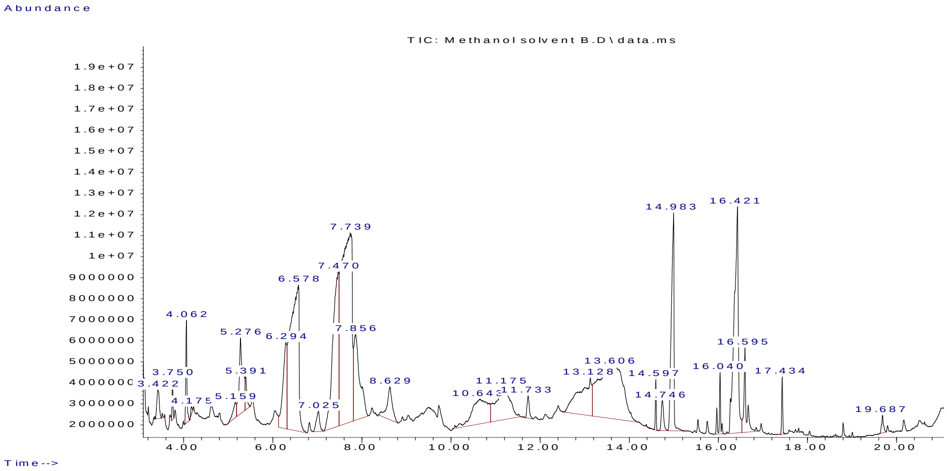
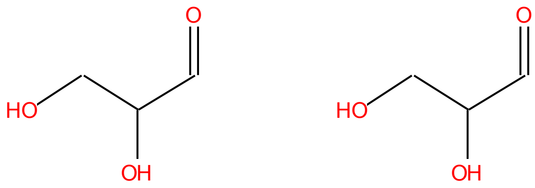
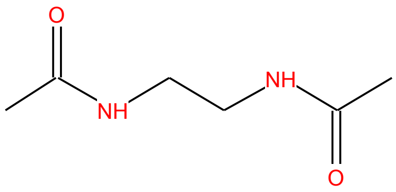
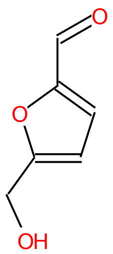
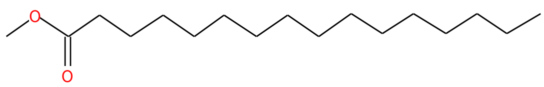
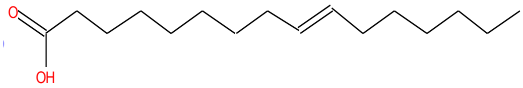
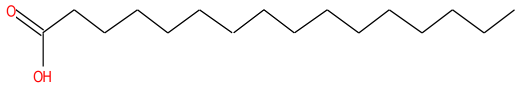
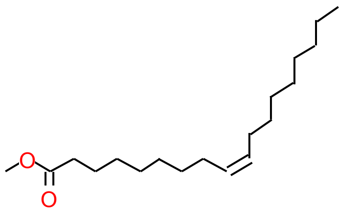
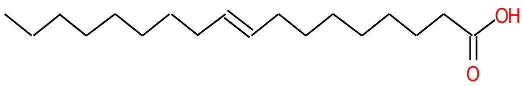
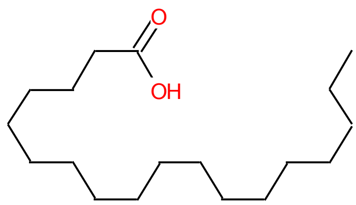
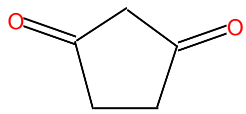
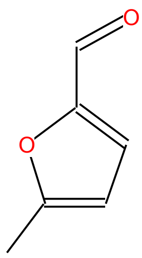
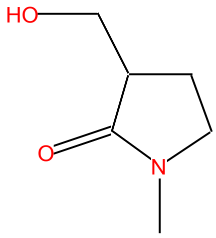


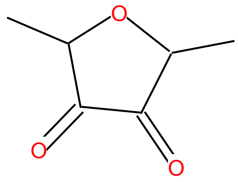
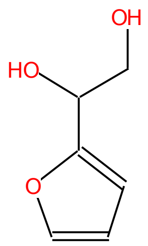
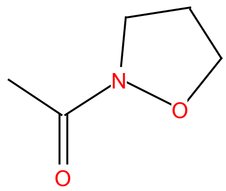
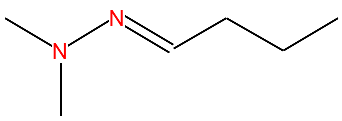
Figure 4.1: Total ion chromatogram (TIC) of the methanol extract of *Azanza garckeana* pulp

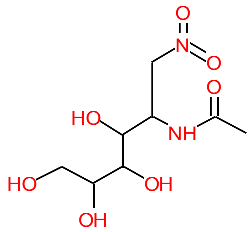
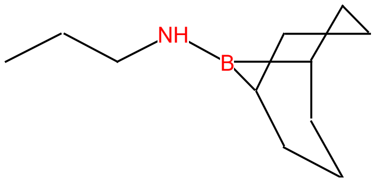
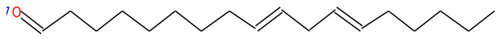
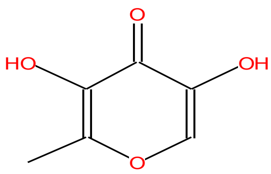
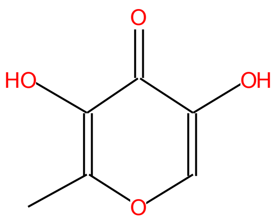
Table 4.8: GC-MS result from the analysis of the methanol extract of *Azanza garckeana* pulp

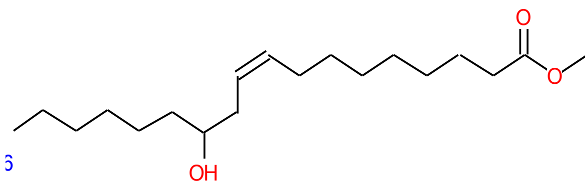
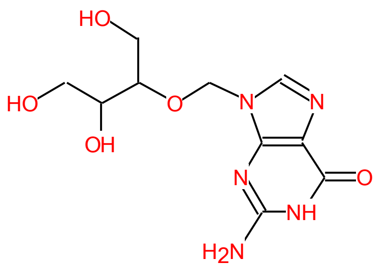
Compound name; chemical formula	**Retention time (min)**	**Area (%)**	**Molecular weight; Chemical structure; Biological activity**
Di-glyceraldehyde dimer $C_6H_{12}O_6$	5.158	0.47	180.16g/mol  Metabolite, substrate for aldose reductase*
N, N'-Di-acetylethylenediamine $C_6H_{16}N_2$	5.392	0.55	116.20g/mol  NF
5-Hydroxy methylfurfural $C_6H_6O_3$	7.468	8.58	126g/mol  Antioxidant, antiproliferative activity*
Hexadecanoic acid, methyl ester $C_{17}H_{34}O_2$	14.597	0.41	270g/mol  Antioxidant, antibacterial, antifungi, hypercholestoerolemic agent, nematicide, pesticide,antiadrogenic agent, flavor, hemolytic agent, 5-Alpha reductase

			inhibitor, potent antimicrobial activity (Akpuaka *et al.*,2013)
9-Hexadecenoic acid $C_{16}H_{30}O_2$	14.744	0.69	254.414g/mol  Antimicrobial, antioxidant*
n-Hexadecanoic acid $C_{16}H_{32}O_2$	14.982	5.33	256g/mol  Anti-inflammatory, Antioxidant, 5-Alpha reductase inhibitor, potent mosquito Larvicide (Majinda and Abubakar, 2016; Ojekale *et al.*, 2016)
9-Octadecenoic acid (Z)-, methyl ester $C_{19}H_{36}O_2$	16.039	0.88	296.50g/mol  Antioxidant activity, anticarcinogenic activity, exist in human red blood cells and serve as endogenous peroxisome proliferator-activated receptor ligand, dermatigenic flavour (Akpuaka *et al.*, 2013)
9-Octadecenoic acid, (E)- $C_{18}H_{34}O_2$	16.420	8.68	282g/mol  Antifungal, Cancer preventive, flavour, anti- inflammatory, anemiagenic, dermatatigenic, 5alpha reductase inhibitor *

Octadecanoic acid $C_{18}H_{36}O_2$	16.597	1.85	284g/mol  Antimicriobal activity*
1,3-cyclopentanedione $C_5H_6O_2$	3.420	0.66	98.1g/mol  NF
2-furancarboxaldehyde-5-methyl $C_6H_6O_2$	3.749	0.36	110.111g/mol  Antimicrobial, preservative *
N-methyl-3-hydroxymethyl pyrrolidin-2-one C_5H_9NO	4.173	0.10	99.133g/mol  NF

2,5-dimethylfuran-3,4 (2H, 5H)-dione $C_6H_8O_3$	5.277	2.63	128.126g/mol  NF
1,2-ethanediol, 1-(2-furanyl) $C_6H_8O_3$	7.854	4.91	128.13g/mol  NF
N-acetylisoxazolidine $C_{11}H_{13}O_4$	8.630	1.63	223.225g/mol  Antioxidant activity, Antibacterial activity (Mukherjee *et al*., 2004)
Butanal, dimethylhydrazone $C_6H_{14}N_2$	10.644	3.78	114.19g/mol  NF
1-nitro-2-acetamido-1,2-dideoxy-d-mannitol $C_8H_{16}N_2O_7$	11.173	4.22	252.22g/mol

			 Antifungal activity (Haider *et al*., 2016)
Propylamine N-(9-borabicyclo [3.3.1]) non-9-yl $C_{11}H_{22}BN$	11.735	0.48	179.11g/mol  Antimicrobial*
9,12-Octadecadienal $C_{18}H_{32}O$	13.606	0.30	264.225g/mol  Antioxidant and antimicrobial activities (Rajeswari *et al*., 2013)
4H-pyran-4-one, 2, 3 dihydro-3,5-dihydroxy-6-methyl $C_6H_8O_4$	6.577	11.99	144.13g/mol  Antioxidant compound, anti-microbial, anti-inflammatory, anticancer, antidiabetic*
4H-pyran-4-one, 2, 3 dihydro-3,5-dihydroxy-2-methyl $C_6H_8O_4$	7.025	0.64	144.13g/mol 

			Antioxidant compound, anti-microbial, anti-inflammatory, anticancer, antidiabetic*
Methyl 12-hydroxy-9-octadecenoate $C_{19}H_{36}O_3$	17.435	0.54	312.5g/mol  NF
2R, 3S -9- [1,3,4-Trihydroxy-2-butoxymethyl] guanine $C_{10}H_{15}N_5O_5$	19.687	12.1 4	285.26g/mol  NF

*source: Dr Duke's Phytochemical and Ethnobotanical database (Duke and Beckstrom, 2008)

** NF: biological activity not found

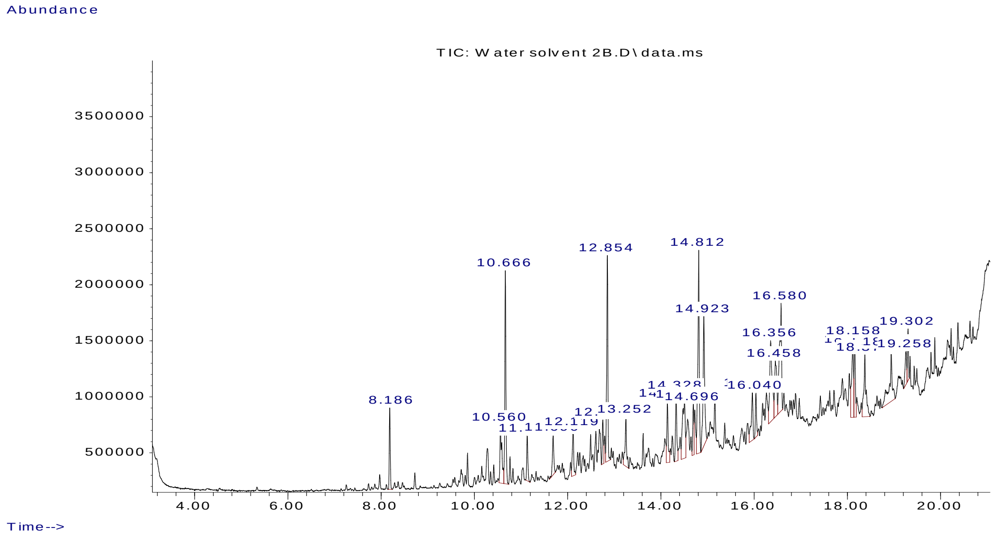
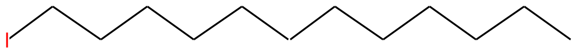
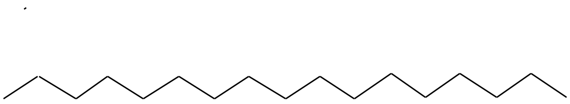
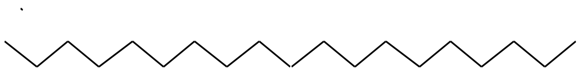
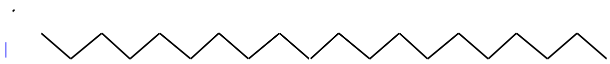
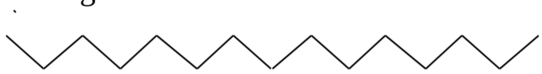
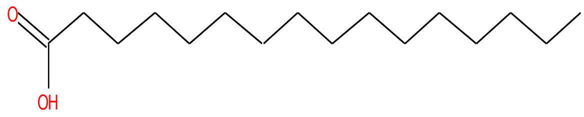
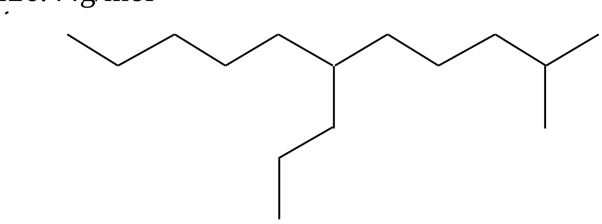
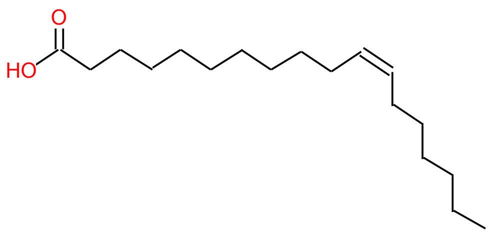
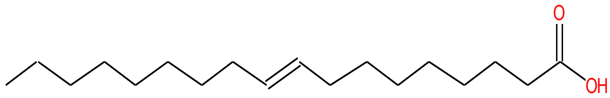
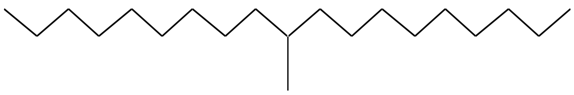
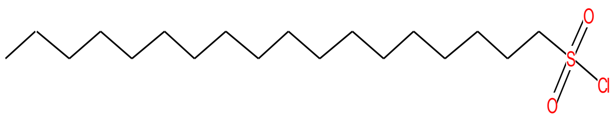
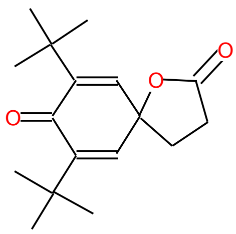
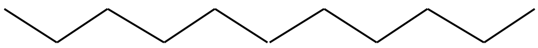


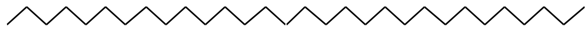
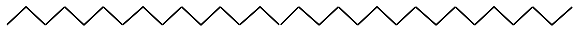
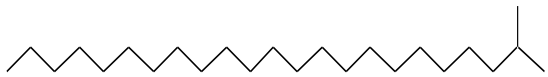
Figure 4.2: Total ion chromatogram (TIC) of the aqueous extract of *Azanza garckeana* pulp

Table 4.9: GC-MS result from the analysis of the aqueous extract of *Azanza garckeana* pulp

Compound name; Chemical formula	Retention time (min)	Area (%)	Molecular weight; Chemical structure; Biological activity
1-iodo, Dodecane ($C_{12}H_{24}I$)	8.187	2.53	296.23g/mol  Antimicrobial*
Octadecane ($C_{18}H_{38}$)	10.558	3.51	254.5g/mol  Antifungal activity against *candidiasis albicans*, antibacterial (Smiline *et al*., 2014)
Eicosane ($C_{20}H_{42}$)	18.944	4.68	282g/mol  Antifungal, antibacterial, antitumor and cytotoxic effects (Hsouna *et al.*, 2011)
Heneicosane ($C_{21}H_{44}$)	10.668	7.57	296g/mol  Antiasthmatic, antimicrobial, and treatment for anemia. (Yogeswari *et al.*, 2012; Babu *et al.*, 2014; Usha *et al.*, 2015)

Hexadecane ($C_{16}H_{34}$)	11.692	1.77	226.44g/mol  Hypercholesterolemic, Antibacterial, antioxidant (Babu *et al.*, 2014)
n-Hexadecanoic acid ($C_{16}H_{32}O_2$)	14.925	6.83	256g/mol  Antioxidant activity, antimicrobial, anti-inflamatory (Jegadeeswari *et al.*, 2012; Majinda and Abubakar, 2016; Ojekale *et al.*, 2016)
2-methyl-6-propyl Dodecane ($C_{16}H_{34}$)	12.754	1.54	226.44g/mol  Antioxidant, Antibacterial, Antimicrobial. (Asnaashari *et al.*, 2016)
Cis-Vaccenic acid ($C_{18}H_{34}O_2$)	16.358	6.37	282.461g/mol  Antibacterial, antifungal, anti-acne, anti-coronary hypolipidemic effects. (Hamazaki *et al.*, 2016).

9-octadecenoic acid ($C_{18}H_{34}O_2$)	16.039	1.61	282.46g/mol  Antioxidant activity, anti-carcinogenic, anti-androgenic (Akpuaka *et al.*, 2013; Arora *et al.*, 2017)
Heptacosane ($C_{27}H_{56}$)	15.964	2.67	380g/mol  Antibacterial, treatment for tetany, anaemia (Mihailovi *et al.*, 2011; Babu *et al.*, 2014)
10-methyl, Eicosane ($C_{21}H_{44}$)	18.158	3.05	296g/mol  Antioxidant*
Heptadecane ($C_{17}H_{36}$)	19.301	1.64	240.5g/mol No biological activity found
1-octadecane sulphonyl chloride ($C_{18}H_{37}ClO_2S$)	18.377	4.29	353g/mol  No biological activity found
7,9-di-tert-butyl-1-oxaspiro (4.5) deca-6,9-diene-2,8-dione ($C_{17}H_{24}O_3$)	14.330	2.85	276.4g/mol  No biological activity found
Dodecane $C_{12}H_{26}$	11.135	1.66	170.33g/mol 

Hentriaconta-ne $C_{31}H_{64}$	13.254	2.53	436.85g/mol 
Nonadecane $C_{19}H_{38}O_2$	14.511	4.91	268.518g/mol 
2- Methyl tetracosane $C_{25}H_{52}$	16.458	3.71	352.7g/mol 

**source: Dr Duke's Phytochemical and Ethnobotanical database (Duke and Beckstrom, 2008)

The compounds found in the methanol extract of the seeds of *Azanza garckeana*, along with their biological activities, retention time (RT), molecular formula, molecular weight and concentration (% Area) are presented in Table 4.10.

4.7.4 GC-MS result from the analysis of the aqueous Extract of *Azanza garckeana* seed

Peaks of the GC-MS compounds detected in the aqueous extract of *Azanza garckeana* seed are shown below in the total ion chromatogram (figure 4.4). A total of 21 bioactive compounds were identified and quantified using their peak and retention time (RT). 9 Octadecenoic acid 12-hydroxymethyl ester (R-(Z))- was found to be the highest in the aqueous extract with 28.042 % in content and RT of 17.472 min, followed by 9,12 Octadecadienoic acid methyl ester (11.78 % in content and RT 15.99 min) while 2,6,11 -trimethyl dodecane was found to be the least with 0.176 % and RT of 8.187 min.

The compounds found in the aqueous extract of the seeds of *Azanza garckeana*, along with their biological activities, retention time (RT), molecular formula, molecular weight and concentration (% Area) are presented in Table 4.11.

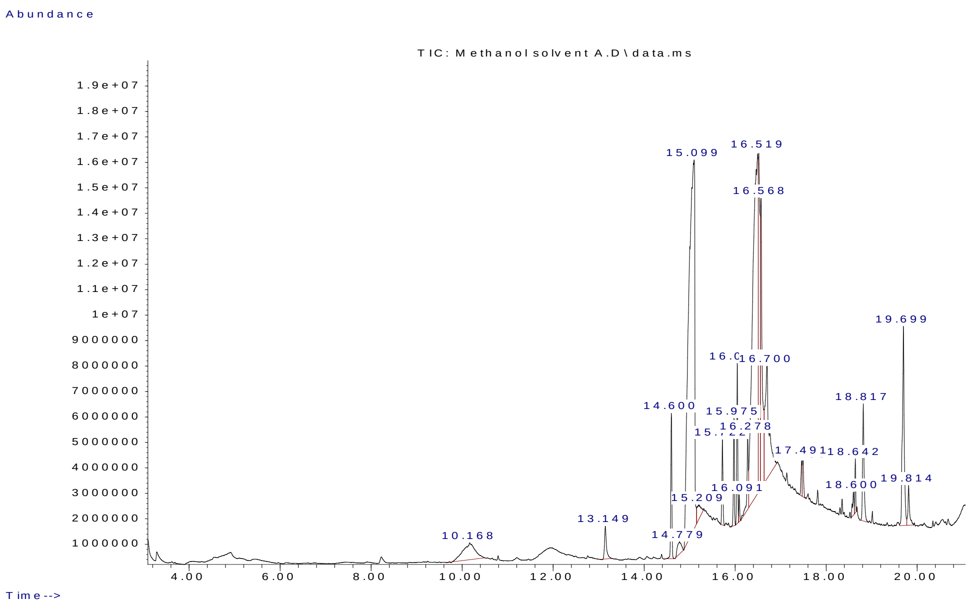
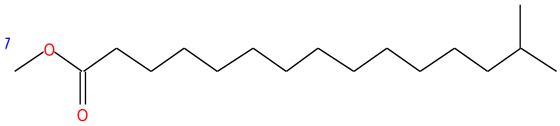
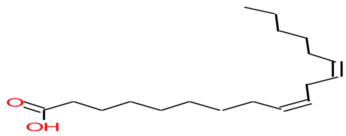
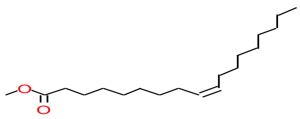
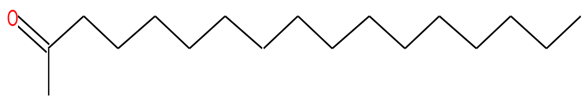
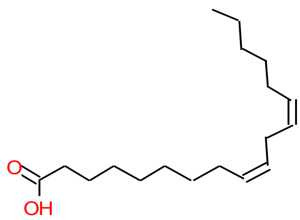
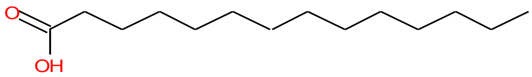
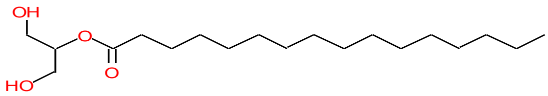
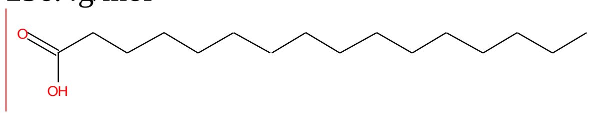
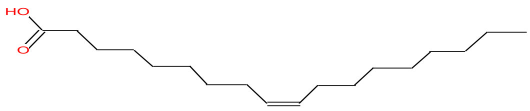
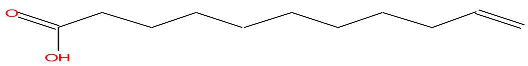
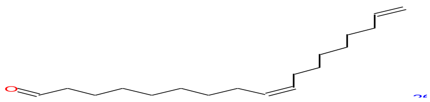
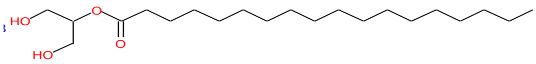
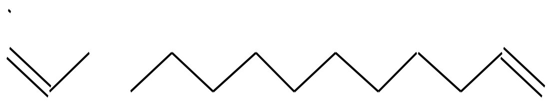
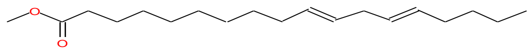
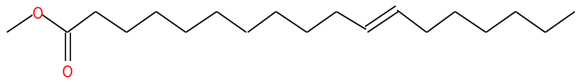
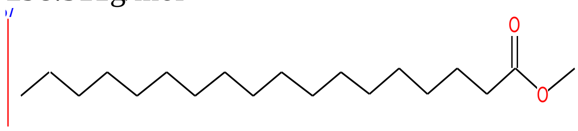
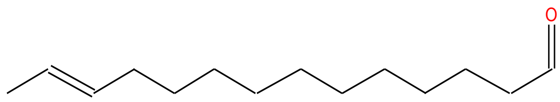


Figure 4.3: Total ion chromatogram (TIC) of the methanol extract of *Azanza garckeana* seed

Table 4.10: GC-MS result from the analysis of the methanol extract of *Azanza garckeana* seed

Compound Name; Chemical Formula	Retention Time (Min)	Area (%)	Molecular Weight; Chemical Structure; Biological Activity
Pentadecanoic acid, 14-methyl-methyl ester $C_{17}H_{34}0_2$	14.601	2.18	270.5g/mol  Antifungal, Antimicrobial (Bashir *et al.*, 2012)
9,12-Octadecadienoic acid (Z,Z)-,methyl ester $C_{19}H_{34}O_2$	15.973	1.42	294.5g/mol  Anticancer (Majinda, and Abubakar, 2016)
9-Octadecenoic acid, methyl ester $C_{19}H_{36}O_2$	16.092	0.33	296.5g/mol  Antioxidant activity, anti- hypertensive, anticarcinogenic activity, exist in human red blood cells and serve as endogenous peroxisome proliferator-activated receptor ligand, increase HDL and decreases LDL. (Akpuaka *et al.*, 2013)
Hexadecanoic acid $C_{16}H_{32}O_2$	15.211	0.76	256.4g/mol  Antioxidant, antimicrobial, anti-inflamatory, haemolytic-5-α reductase inhibitor, antiandrogenic activities (Majinda and Abubakar, 2016; Ojekale *et al.*, 2016)
9,12 – octadecadienoic acid-(z-z) $C_{18}H_{32}O_2$	16.497	24.3	280.5g/mol  Hepatoprotective, antihistaminic,

			hypocholesterolemic, antieczemic, anticancer and anti-inflammatory properties (Majinda and Abubakar, 2016; Sunita *et al.*, 2017)
Tetradecanoic acid $C_{14}H_{28}O_2$	13.149	0.854	228.4g/mol  Antifungal, antioxidant, hypercholesterolemic, cancer preventive, nematicide, lubricant*
1-hydroxymethyl, 2-hydroxy-Hexadecanoic acid ethyl ester $C_{19}H_{38}O_4$	18.816	2.37	330.5g/mol  Antioxidant, hypercholesterolemic, nematicide, pesticide, antiandrogenic activities, haemolytic-5-α reductase inhibitor*
n-hexadecanoic acid $C_{16}H_{32}O_2$	15.211	0.76	256.4g/mol  Antioxidant, anti-inflammatory properties, antitumor *
Oleic acid $C_{18}H_{34}O_2$	16.568	7.04	282.5g/mol  Antifungal activity *
Undecylenic acid $C_{11}H_{20}O_2$	17.463	0.51	184g/mol  Antifungal activity*
9, 17 octadecadienal (Z) $C_{18}H_{32}O$	19.701	4.97	264.5g/mol  Antimicrobial, anti-inflammatory*
1-hydroxymethyl, 2-hydroxy-Octadecanoic acid ethyl ester $C_{21}H_{42}O_4$	19.816	0.88	358.56g/mol  Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic, anticancer, anticoronary, antiacne and anti-inflammatory properties* (Majinda and Abubakar, 2016)

1,13-Tetradecadiene $C_{14}H_{26}$	15.72	1.24	194g/mol  NF
10,13-Octadecadienoic acid, methyl ester $C_{19}H_{34}O_2$	15.97	1.42	294.5g/mol  NF
11-Octadecenoic acid, methyl ester $C_{19}H_{36}O_2$	16.05	2.15	297g/mol  Antibacterial, antifungal, antioxidant (Asghar and Choudahry, 2011)
Methyl stearate	16.28	1.40	298.511g/mol  White crystal semi-solid ester; flavour component in food; Lubricant; used in the manufacture of pharmaceuticals, cosmetics and soaps; Surfactant and softening agent (Enas and Duha, 2014)
E-12-Tetradecenal $C_{14}H_{26}O$	17.49	0.66	210g/mol  NF

*source: Dr Duke's Phytochemical and Ethnobotanical database (Duke and Beckstrom, 2008)

NF: Biological activity not found

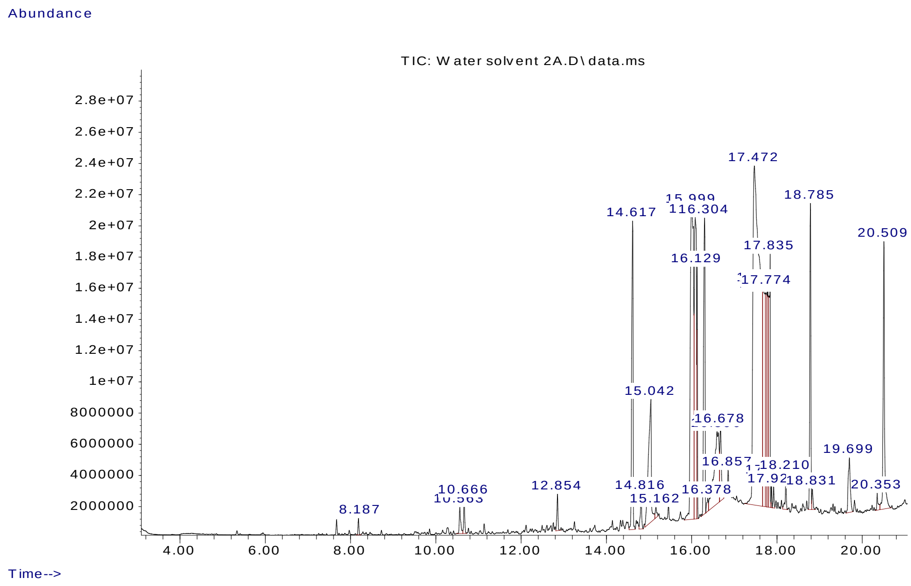
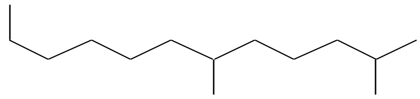
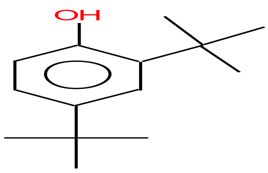
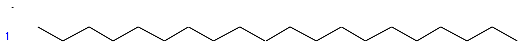
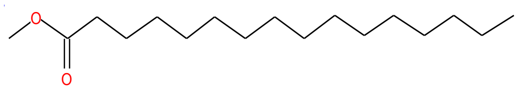
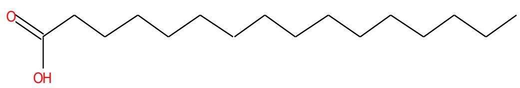
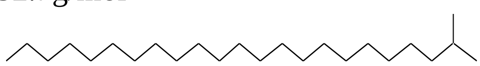
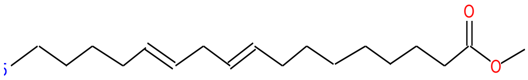
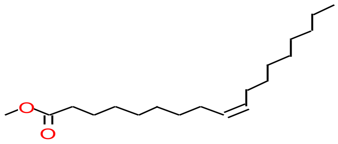
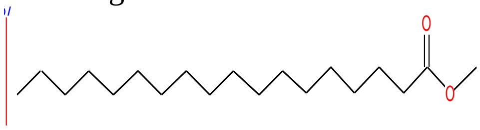
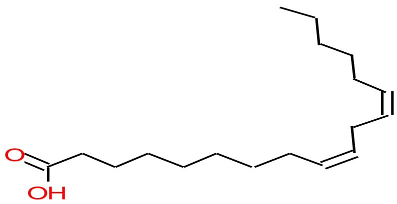
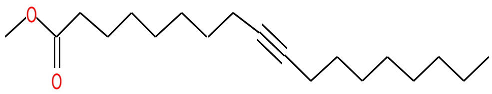
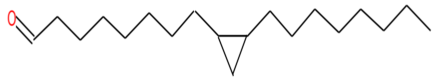
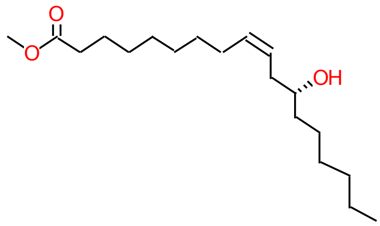
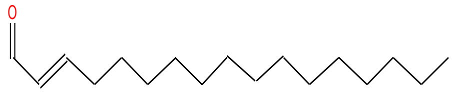
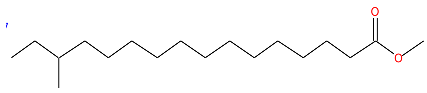
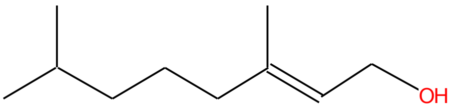
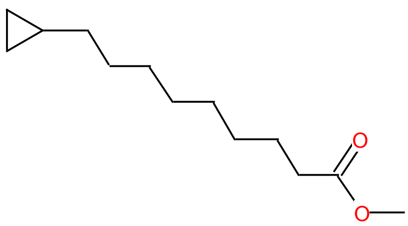


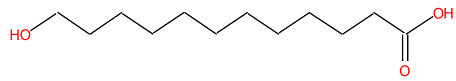
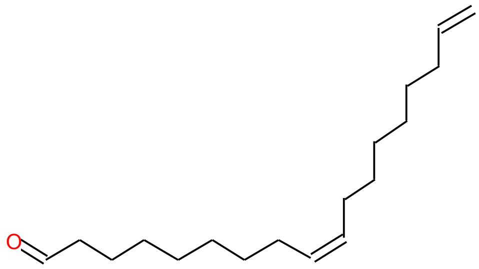
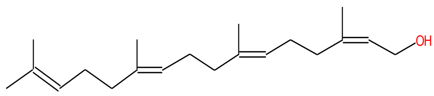
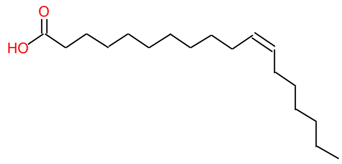
Figure 4.4: Total ion chromatogram (TIC) of the aqueous extract of *Azanza garckeana* seed

Table 4.11: GC-MS result from the analysis of the aqueous extract of *Azanza garckeana* seed

Compound Name; Chemical Formula	Retention Time (Min)	Area (%)	Molecular Weight; Chemical Structure; Biological Activity
Dodecane, 2,6,11-trimethyl- $C_{15}H_{32}$	8.187	0.18	212.41g/mol  Antibacterial activity *
2,4-Di-tertbutylphenol $C_{14}H_{22}O$	10.563	0.44	206.32g/mol  NF
Heneicosane $C_{21}H_{44}$	14.816	0.55	296.57g/mol  Antiasthmatics, urine acidifiers, antimicriobal *
Hexadecanoic acid, methyl ester $C_{17}H_{34}O_2$	14.616	5.03	270.453g/mol  Antioxidant, antimicrobial, anti-inflamatory, haemolytic-5-α reductase inhibitor, antiandrogenic activities (Majinda and Abubakar, 2016; Ojekale *et al.*, 2016).
n-Hexadecanoic acid $C_{16}H_{32}O_2$	15.044	4.25	256.4g/mol  Antioxidant, Hypocholesterolmic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor activities.*
2-Methyltetracosane $C_{25}H_{52}$	15.163	0.18	352.7g/mol  Drug for dermatological disorders, antibacterial and urinary problem*

9,12-Octadecadienoic acid, methyl ester* $C_{19}H_{34}O_2$	15.996	11.8	294.472g/mol  Antibacterial*
9-Octadecenoic acid (Z)-, methyl ester	16.087	7.34	296g/mol  cancer preventive (Simin *et al.*, 2000), anti-inflammatory (Yunfeng *et al.*, 2007) and antimicrobial activity (Wagh *et al.*, 2007)
Methyl stearate $C_{19}H_{38}O_2$	16.306	4.82	298.511g/mol  White crystal semi-solid ester; flavor component in food; Lubricant; used in the manufacture of pharmaceuticals, cosmetics and soaps; Surfactant and softening agent (Enas and Duha, 2014).
9,12-Octadecadienoic acid (Z, Z); $C_{19}H_{34}O_2$	16.596	4.29	294g/mol  Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic, anticancer and anti-inflamatory properties (Majinda and Abubakar, 2016).
9-Octadecynoic acid, methyl ester $C_{19}H_{34}O_2$	16.38	0.24	294.5g/mol  NF

Cyclopropane octanal, 2-octyl- $C_{19}H_{36}O$	16.86	0.28	280.5g/mol  NF
9-Octadecenoic acid, 12-hydroxy- methyl ester, [R-(Z)]- $C_{19}H_{36}O_3$	17.47	28.0	312.5g/mol  NF
2-Heptadecenal $C_{17}H_{32}O$	17.87	0.20	252.4g/mol  NF
Hexadecanoic acid, 14-methyl-, methyl ester $C_{18}H_{36}O_2$	17.93	0.22	284.5g/mol  NF
2-Octen-1-ol, 3,7-dimethyl- $C_{10}H_{20}O$	18.21	0.46	156.26g/mol  NF
Cyclopropanenonanoic acid, methyl ester $C_{13}H_{24}O_2$	18.79	4.04	212.33g/mol  NF

12Hydroxydodecanoic acid $C_{12}H_{24}O_3$	18.83	0.27	216.32g/mol  NF
9,17-Octadecadienal, (Z)- $C_{18}H_{32}O$	19.70	1.34	264.4g/mol  NF
Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl- $C_{20}H_{34}O$	20.35	0.27	290.5g/mol  NF
cis-Vaccenic acid $C_{18}H_{34}O_2$	20.511	4.48	282.5g/mol  NF

*source: Dr Duke's Phytochemical and Ethnobotanical database (Duke and Beckstrom, 2008)

NF: Biological activity not found

CHAPTER FIVE

DISCUSSION

Azanza garckeana is an indigenous wild fruit tree with increasing interest in it use for several purposes. This study examined the effect of aqueous-methanol pulp extract on biochemical, haematological and histopathological parameters in male Wistar albino rats. Its effect on sex hormone were also evaluated and medicinal properties of the fruits ascertained using gas chromatography-mass spectrometry (GC-MS).

Plasma lipid levels that are too high are a risk factor for heart disease (Owolabi *et al.*, 2010). In this study, assessment of LDL, HDL, Total Cholesterol, and TAG revealed no significant alteration ($p > 0.05$) in LDL, HDL, cholesterol and Triacylglycerol concentration. This result indicates that the aqueous-methanol pulp extract of *Azanza garckeana* may possess hypolipidemic effect. This finding is consistent with Agoreyo *et al*. (2017), who found a non-significant difference in lipid profile in Wistar albino rats administered aqueous-methanol leaves extracts of two *Adenia lobata* varieties.

The enzymes; Alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and bilirubin are useful in the detection of liver damage (Singh *et al.,* 2011). When the liver cells are damaged or injured, the cells secrete these enzymes into the bloodstream, causing the enzyme levels to rise (Viriyavejakul *et al*., 2014). Consequently, an increase in the activities of serum enzymes is considered a vital indicator of liver toxicity. The result obtained from this study showed no significant difference ($p > 0.05$) in enzyme activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the test groups relative to control (Table 4.4). This along with histopathology findings of the liver cells shown in the photomicrographs presented in plate 4.1-4.4 clearly indicates that the aqueous-methanol extract of *Azanza garckeana* has no deleterious effect on the integrity of the liver. This finding is consistent to the non-significant difference observed by Diallo *et al*. (2010) in the levels of ALT, AST and alkaline phosphatase in the control and test groups administered hydroalcoholic extracts of *Ageratum conyzoides* for twenty-eight (28) days.

Bilirubin which is derived from the breakdown of haemoglobin present in red blood cells is circulated in the blood. During normal function, bilirubin is mopped up by the liver and excreted through bile as bile pigment. An increase in the levels of total bilirubin may indicate a failure in the liver's ability to excrete bilirubin or an obstruction in the bile duct (Veena *et al.,* 2012). The result obtained from this study showed a non-significant decrease ($p > 0.05$) in both total and conjugate bilirubin concentration for all test groups compared with the control group (Table 4.4). This indicates that the ability of the liver to excrete bilirubin is not compromised by the administration of the aqueous-methanol pulp extract of *Azanza garckeana.* This result is in agreement with that reported by Okonkwo *et al*. (2010), who showed that the methanol extract of the inner stem bark of *A. occidentale* stimulated an insignificant difference in total bilirubin level.

Evaluation of total protein is also one of the measures used in detection of liver function. Total protein refers to the combined amount of albumin and globulin in the blood. Albumin is a pigment that prevents fluid from leaking out of blood vessels, and globulins are a crucial component of the immune system. Result from this study revealed a non-significant difference ($p > 0.05$) in total protein concentration in the three test groups compared with control group (Table 4.4). The non-significant difference in total protein may be indicative that the extract did not alter the liver function. This result is in agreement with that reported by Agoreyo *et al.* (2017) who showed that the aqueous-methanol extract of two varieties of *Adenia lobata* has no significant effect on total protein levels.

Albumin is produced in the liver and a decrease in its level is consistent with hepatotoxicity. The result obtained from this study revealed a non-significant increase ($p > 0.05$) in Albumin indicating that the extract has no deleterious effect on hepatocellular function. This result is in agreement with that reported by Agoreyo *et al.* (2017), which showed that the aqueous-methanol extract of two varieties of *Adenia lobata* has no significant effect on Albumin levels.

The kidney has important influence on drug metabolism and it is susceptible to toxicants because of a high volume of blood that flows through it (Lawal *et al.*, 2015). The function of the kidney is measured by its capacity to effectively remove toxic waste product from the blood and regulate plasma electrolyte. The evaluation of creatinine, urea and electrolytes levels play important roles in determining the synthetic and excretory roles of the kidney and may assist in diagnosis of kidney impairment (Onukogu *et al.,* 2019).

Creatinine is a catabolic breakdown product that is synthesized from creatine and phosphocreatine in skeletal muscles. The human body produces creatinine at a relatively stable rate (depending on muscle mass), and it has been shown to be a reasonably accurate measure of kidney activity. Creatinine levels above a certain threshold indicate reduced kidney function or kidney failure (Davis *et al*., 2018). Any form of impediment to the kidney will result in rise in creatinine level in the blood, due to inability of the kidney to clear creatinine.

In this study, a significant increase in creatinine levels at 300 and 2000 mg/kg bodyweight was recorded (Table 4.5). However, the significant increase ($p < 0.05$) in serum creatinine levels in the 300 and 2000 mg/kg body weight test groups may not be attributable to renal impairment, as the histopathology photomicrographs presented in plates 4.5-4.8 clearly rule out renal impairment. This finding is in disagreement with the findings of Osagie-Eweka *et al.* (2019) who reported a non-significant change in creatinine levels in rats administered methanol leaf extract of *Simarouba glauca*.

Urea is a by-product of purine and protein catabolism (Watford, 2003). It circulates through the bloodstream until the kidneys flush it out and excrete it in urine. When urea levels surpass permissible limits, it is considered toxic (Latha *et al.*, 2016). Elevated levels of urea in the blood stream are recorded if the kidney function is impaired. In this study, no significant change ($p > 0.05$) in urea concentration level was observed indicating that the pulp extract of *Azanza garckeana* did not elicit significant renal impairment capable of limiting the kidney's excretory function. This finding is in disagreement with the findings of Osagie-Eweka *et al.* (2019) who reported a significant change in urea levels in rats administered methanol leaf extract of *Simarouba glauca*.

Changes in blood electrolyte levels is an indication of fluid imbalance and can be used to diagnose the presence of kidney failure. Sodium ion (Na^+) is a major cation of the extracellular fluid and contributes to the regulation of body fluid and osmotic pressure at the extracellular and intracellular fluid compartments. Chloride ion (Cl^-) is a major anion of the extracellular fluid compartment that contributes to electrolyte regulation and balance including proper hydration, osmotic pressure and acid/base balance (Wu, 2006). Potassium ion (K^+) is also a vital cation of the intracellular fluid compartment and it is also an important constituent of the extracellular fluid due to its influence on muscle activity, acid-base regulation and osmotic pressure in body fluids (Osagie-Eweka *et al*., 2019). Increase level of potassium is often associated with kidney derangement, dehydration shock or even adrenal insufficiency (Wu, 2006). The result obtained from this study (Table 4.5) shows no significant change ($p > 0.05$) in sodium, Potassium and chloride ion which indicate that the electrolyte regulatory function of the kidneys was not compromised by oral administration of aqueous-methanol pulp extract of *Azanza garckeana*. The findings for Na^+ and K^+ ions levels is contrary to the findings of Osagie-Eweka *et al.* (2019) who reported significant change in Na^+ and K^+ ion levels in rats administered methanol leaf extract of *Simarouba glauca.*

Plasma HCO3- ion exist in plasma or serum as dissolved CO_2 and the bicarbonate anion also serves as physiological buffering system to maintain the internal environment regulated by the kidney. Plasma bicarbonate ion is elevated in compensatory response to metabolic alkalosis and respiratory acidosis whereas, it is lowered in response to metabolic acidosis and respiratory alkalosis respectively (Burtis and Ashwood 2000). The result obtained from this study indicate no significant change ($p > 0.05$) in the bicarbonate levels across the test groups compared to control (Table 4.5). The finding for HCO3- ions levels is similar to the findings of Osagie-Eweka *et al.* (2019) who reported a non-significant change in HCO3- ions levels in rats administered methanol leaf extract of *Simarouba glauca.*

The blood-related functions of plant extract can be determined using haematological parameters. In both humans and animals, the haemopoietic pathway is one of the most sensitive targets for toxins and a significant indicator of physiological and pathological status. The extract induced a non-significant difference on the RBC count which suggests that it has no effect on erythropoiesis, morphology, or osmotic fragility of red blood cells (Porwal *et al*., 2017). WBCs are the first line of defence in the body, responding to infectious agents, tissue damage, and inflammation. When compared to the control sample, the white blood cell (WBC), lymphocyte, and monocyte counts of the tests samples were not substantially different ($p > 0.05$)

(Table 4.6). This suggests the extract had little or no negative impact on the rats' immune system.

Red blood cell distribution width (RDW) is a metric that depicts the spectrum of red blood cell size and volume difference. Elevated RDW is used alongside mean corpuscular volume (MCV) to diagnose anaemia. In this study the MCV in the test groups was not significantly altered compared with control while those of the RDW were significantly lowered relative to the control (Table 4.6). Therefore, this implies that aqueous methanol pulp extract of *Azanza garckeana* fruits has no deleterious effect on the red blood cell size and volume as low RDW is desirable. The haematological results in this analysis are consistent with Prasanth *et al*. (2015) findings of a non-significant difference in haematological parameters in the control and treatment groups after 4 weeks of administration of ethanol root extracts of *Oncoba spinosa.*

Histopathological findings from this study revealed no alteration in the organs; liver, kidney, heart, colon and testis of male Wistar albino rats administered aqueous-methanol pulp extract of *Azanza garckeana* (Plate 4.1- 4.20). These findings indicate that the plant extract did not compromise the cellular integrity nor inflict damage to these organs. This result is in agreement with that reported by Agoreyo *et al.* (2017), which showed that the aqueous-methanol extract of two varieties of *Adenia lobata* had no significant effect on histology of the liver, kidney, heart and colon.

Testosterone is the main androgen hormone that is found in males and it is produced by the interstitial cells of the leydig in the testis. This study showed a non-significant change ($p > 0.05$) in testosterone level indicating that the extract had no effect on testosterone levels in the rats (Table 4.7). This finding is contrary to the findings of Chukwunonso and Olusanya (2017) who reported a significant decrease in testosterone in male rats administered aqueous extract of *Hibiscus saddariffa* calyx. Luteinising hormone and FSH are required for the initiation and maintenance of spermatogenesis in pubertal rats (Yakubu *et al.,* 2007). The non-significant effect observed in FSH and LH (Table 4.7) may be because the hypothalamic-pituitary axis was not affected by the extract, and can be suggestive that the plant extract neither possesses pro-gonadotrophic nor anti-gonadotrophic effect in the male Wistar albino rats. This finding is similar to the findings of Yakubu *et al.* (2007) who reported a non-significant change in serum concentrations of FSH and LH in male rats administered aqueous extract of *Chromolaena odoratum leaves*.

Progesterone which is an endogenous steroid plays an important role as a metabolic intermediate in the synthesis of other endogenous steroid such as testosterone. In this study progesterone levels were increased significantly ($p < 0.05$) in the groups administered doses of 300 and 2000 mg/kg body weight of the extract. This finding indicates that the administration of aqueous-methanol extract of *Azanza garckeana* may play a role in enhancing the production of testosterone through the increase in progesterone levels, which can serve as an intermediate for testosterone synthesis. This finding is contrary to the findings of Okereke and Onuoha (2015) who reported a significant decrease in progesterone in Wistar rats administered ethanolic extract of *cannabis sativa.*

Prolactin although found in low amount in males but plays important physiological role by enhancing luteinizing hormone receptors in leydig cells, which result in testosterone secretion, leading to spermatogenesis (Hair *et al.,* 2002). Prolactin levels in the three test groups was found to be non-significantly different compared with the control (Table 4.7). Result from this study also revealed a non-significant decrease in the estradiol levels of male Wistar albino rats. The prolactin and estradiol finding is contrary to the findings of Chukwunonso and Olusanya (2017) who reported a significant decrease in prolactin and estradiol in male rats administered aqueous extract of *Hibiscus saddariffa* calyx.

Plants with medicinal properties are of great benefits to the health and wellbeing of individuals. These medicinal plants are of great interest to researchers, in order to identify important bioactive compounds that may be useful in the production of drugs (Ncube *et al.*, 2008). Therefore, in a bid to evaluate the medicinal properties of the fruit of *Azanza garckeana*, the bioactive compounds present in both the pulp and seeds of *Azanza garckeana* fruit were identified and quantified using Gas Chromatography Mass Spectrometric (GC-MS) technique.

The GC-MS chromatogram of the methanol extract of *Azanza garckeana* (Figure 4.1) showed twenty-three (23) peaks indicating the presence of 23 bioactive compounds. A number of twenty-three (23) bioactive constituents were characterized and identified on comparison of the mass spectra of the constituent with the NIST library. The compounds 5-hydroxymethylfurfural (19.39 %), 2R, 3S-9-[1,3,4-Trihydroxy-2-butoxymethyl] guanine (12.14 %) and 4-H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (11.99 %) were the primary compounds identified in the methanol extract, while N-methyl-3-hydroxymethyl pyrollidin (0.095 %) was the least. The compound 5-hydroxymethylfurfural (5-HMF) is reported to possess antioxidant and anti-proliferative activities (Dr. Dukes Phytochemical and Ethnobotanical databases). A prelude *in vivo* studies carried out by Abdumalik *et al*. (2005), using transgenic sickle mice reported that orally administered 5-HMF inhibits sickled cells formation in the blood.

The aqueous extract of the pulp of *Azanza garckeana* fruits revealed the presence of eighteen (18) bioactive compounds (Figure 4.2). The prevailing compounds that were found in the aqueous extract were heneicosane (9.00 %), octadecane (7.041 %), hexadecenoic acid (6.82 %) and cis-vaccenic acid (6.37 %), while Dodecane, 2-methyl,6-propyl was the least component (1.54 %) identified. Heneicosane is reported to be a potent anti-asthmatic, antimicrobial and anti-anaemic agent (Usha *et al*., 2015; Babu *et al*., 2014; Yogeswari *et al.,* 2012). n-Hexadecanoic acid also identified in the pulp has being reported to exhibit antioxidant, anti-inflammatory and antimicrobial activities (Majinda and Abubakar, 2016; Ojekale *et al*., 2016; Jegadeeswari *et al.,* 2012). Many of the identified bioactive compounds have been reported to have antioxidant property (Table 4.8 and 4.9). The antioxidant compounds play important roles in the prevention of free radical formation and thus help in lowering the risk of chronic and degenerative ailments. Also Cis vaccenic acid, which is an omega-7-fatty acid present in the pulp is known to exhibit antibacterial, antifungal, anti-acne effects (Hamazaki *et al*., 2016). Octadecane appears to exhibit antibacterial activity and also possess antifungal activity against *candidiasis albicans* (Smiline *et al*., 2014).

The GC-MS analysis of the methanol extract of *Azanza garckeana* seeds also revealed several compounds with varying biological activities. The compound hexadecanoic acid was found to be the highest in the methanol extract (Table 4.10) and is reported to have antioxidant property (Ojekale *et al.,* 2016). Antioxidant compounds are known to help prevent cancer, as extensive laboratory data from chemical, cell culture, and animal research has shown that antioxidants can delay or even prevent cancer growth by scavenging free radicals. Other compounds in the methanol extract of *Azanza garckeana* seeds that were found to have antioxidant properties include; 9-Octadecenoic acid methyl ester, Tetradecanoic acid, 11-Octadecenoic acid methyl ester, Hexadecanoic acid, and 2 hydroxyl-1-(hydroxymethyl) ethyl ester. The compounds Oleic acid, Undecylenic acid,2mono Stearin, 9, 17-octadecadienal, and 14-methyl-methyl Pentadecanoic acid ester identified in the methanol extract of the seeds of *Azanza* *garckeana* have been reported to have antimicrobial activities. The antimicrobial effects of these bioactive compounds maybe due to the fact that they are fatty acids, fatty acid ester and aliphatic chains (long chain alkanes and alkenes). These compounds can accumulate in the lipid bilayer of the cell membrane and mitochondria. Consequently, disturbing the integrity of the cell structure of these microbes and causing it to become more fluid and thus increasing their membrane permeability (Solorzano-Santos and Miranda-Novales, 2012). The increased permeability of the membrane by the insertion of unsaturated medium- and long-chain free fatty acids can allow internal contents to leak from the cells of these microbes, which can cause growth inhibition or even death. Also increase in membrane permeability can inhibit enzyme activity that is crucial for survival and growth of these microbes in the membrane or cytosol (Zheng *et al.,* 2005).

The following compounds namely 9-octadecenoic acid 12 hydroxylmethyl ester, 9,12 octadecadienoic acid methyl ester and 9 octadecenoic acid methyl ester were found to be the major compounds in the aqueous extract of the seed of *Azanza garckeana* with 28.04 %, 11.78 % and 7.34 % concentration respectively (Table 4.11). The compounds 9,12 octadecadienoic acid methyl ester and 9 octadecenoic acid methyl ester have being reported to have antibacterial, anti-inflammatory and antimicrobial activities (Table 4.11). Other minor constituents present in the aqueous seed extract of *Azanza garckeana* are hexadecanoic acid (4.25 %), hexadecanoic acid, methyl ester (5.03 %), methyl stearate (4.82 %), methylinoleate (4.29 %), 12- hydroxydodecanoic acid (0.27 %), 2,6,11- trimethyldodecane (0.18 %) with various biological activities as shown in Table 4. The majority of the compounds contained in the aqueous extract have been confirmed to have intriguing biological functions, with some of them being similar to those found in the methanol extract. The compounds 2,6,11 trimethyl Dodecane, heneicosane, hexadecanoic acid, hexadecanoic acid methyl ester have antimicrobial activity and may carry out this function as described previously for compounds that were found in the methanol extract with similar biological activity. Hexadecanoic acid and hexadecanoic acid methylester found in the aqueous extract also have antioxidant, and anti-inflammatory properties. The discovery of many bioactive compounds in *Azanza garckeana* fruit supports their ethnomedical applications and indicates that the plant has therapeutic properties that could be used for health benefits.

5.1 Conclusion

The results of this study show that the aqueous-methanol pulp extract of *Azanza garckeana* has no negative impact on the biochemical, haematological, or histopathological parameters studied. The aqueous-methanol pulp extract also has an effect on the sex hormone progesterone and in addition, the fruit of *Azanza garckeana* plant has medicinal properties, which gives credence to their ethnomedicinal use.

5.2 Contribution to Knowledge

The study has contributed to knowledge in the following ways:

1. This study established that the pulp of *Azanza garckeana* fruits has no adverse effect on the functions of the liver and kidney, as well as lipid profile in male Wistar albino rats.
2. This study also revealed that the pulp of *Azanza garckeana* fruits has decreasing effect on the platelet count, platelet crit and RDW value in male Wistar albino rats.
3. This study also reveals that the pulp of the fruits of *Azanza garckeana* increases the level of the sex hormone, progesterone in male Wistar albino rats.

4. The study also established that the oral administration of aqueous-methanol pulp extract of *Azanza garckeana* fruits has no adverse effect or damage on the liver, kidney, colon, heart and testes of male Wistar albino rats.
5. Several Bioactive compounds with varying medicinal properties present in both the pulp and seeds of *Azanza garckeana* fruits were identified and quantified using GC-MS analysis, for the first time.

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APPENDIX I

CALCULATION OF EXTRACT DOSE

Group: 50mg/kg b.wt

50mg → 1000g of rat

Xmg → 213.8g

$= \frac{50 \times 213.8}{1000} = 10.69\text{mg}$

Group: 300mg/kg b.wt

300mg → 1000g of rat

xmg → 213.8g

$= \frac{300 \times 213.8}{1000} = 64.14\text{mg}$

Group: 2000mg/kg b.wt

2000mg of extract → 1000 g rat

x → 180g rat

$= \frac{2000 \times 180}{1000}$ = 360mg of extract

Each of the dose calculated were simply dissolved in 1ml distilled water and administered

APPENDIX II

Manual calculation for ALT

Absorbance	U/l	Absorbance	U/l
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.375	67
0.150	25	0.400	72
0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

Manual calculation for AST

Absorbance	U/l	Absorbance	U/l
0.020	7	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59
0.070	23	0.150	67
0.080	27	0.160	76
0.090	31	0.170	89