

**THE EFFECT OF WATER EXTRACT OF COLA NITIDA POD
ON LIPOPROTEIN CONCENTRATIONS OF ALBINO
WISTAR RATS**

BY

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CETIFICATION

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DEDICATION

To the Almighty God; and to everyone who has influenced my life positively.

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First amongst all, my gratitude goes to God for giving me life, wisdom and knowledge which I applied in this work. My primary obligation goes to my immediate family, my parents Mr. and Mrs. Mike Oguike, my sister and brother, Chinwe and Chibueze Oguike for their love and support.

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ABSTRACT

Cola nitida has been in use in the eastern parts of Nigeria for the management of certain ailments and as an aphrodisiac in the management of some sexual dysfunctions. However the effects of this extract in some health parameters in human objects have not been reported. This work, then, was aimed at investigating the effect of water extracts of kola pods on lipoprotein concentrations on albino wistar rats.

Rats were used in this study and they were divided into several groups and fed with a high calorie food (cow's brain) so as to increase the blood lipoprotein concentration in the rat models. The effect of the water extracts of the sample used was compared with a known drug and the results statistically compared to ascertain the effect of the water extract on the rat models.

The results of this work indicate that water extracts of cola nitida pods can be used in the management of mild lipoprotein dysfunctions thus substantiating the reasons for its use in sexual erectile functions.

CHAPTER ONE

1.1 INTRODUCTION

Cola Nitida has been used in folk medicine as an aphrodisiac, an appetite suppressant, to treat morning sickness, migraine headache, and indigestion (Esimone *et al.*, 2007). It has also been applied directly to the skin to treat wounds and inflammation (Newall *et al.*, 1996) on the teeth and gums. There has also been speculations as to its use in the treatment of peptic ulcer, however, in Nigeria there is no record of the incidence of peptic ulcer.

According to Esimone (2007), cola nitida tree is native to West Africa. Cola nuts are obtained from cola trees. Cola nitida belongs to the genus cola and family *steriliaceae*. They are commonly used to counteract hunger and thirst; in some cases it is used to control vomiting in pregnant woman and also as a principal stimulant to keep awake and withstand fatigue by students, drivers, and other menial workers.

Lipoproteins are found in the liver and cells. The liver is the largest organ inside the body. The liver is dark reddish-brown and

consists of two main lobes. There are over 300 billion specialized cells in the liver that are connected by a well organized system of “bile” ducts and blood vessels called the biliary system.

CHAPTER TWO

LITERATURE REVIEW

Cola husk pod has great potentials as feed ingredients (Hamzat *et al.*, 2003). Cola tree is a tropical tree which belongs to the family of *steriliaceae*. It is mostly common in the rain forest region of West Africa. This crop is of socio-economic importance; there is a variety of kola species, out of which *cola nitida* and *cola accuminata* are of major economic and social importance in Nigeria. (Babafunde *et al.*, 2001.)

According to Russelu (2005) the tree is a robust tree usually from 12m high. In South Western Nigeria where large plantation is established, I discovered that *cola nitida* pod husk shares several similarities with coco pod husk; both have high crude protein and low crude fibre content. Kola pod husk contains the highest value of calcium (0.60%), chloride ion (0.44%) and molybdenum (9.4ppm) (lowest value of this nutrient is observed in kola test (0.7ppm). The seeds of both *cola nitida* and *cola accuminata*, its nutritive quality have been reported cogutuga.

2.1 KOLA POD COMPONENTS

The kola pod components were found to have supportive effect on fat accumulation. Sayami *et al.*, 2010, described that 2% caffeine powder suppresses body weight gain and fat accumulation. It also reduces triacylglycerol (TG) and total cholesterol (TC) in the liver and as well as (TG) in serum, without reducing food intake (in mice). As a result, it was shown that the combination of caffeine and catechin might be the most effective of all green tea components in suppressing fat accumulation. Body fat gain in mice fed with a high-fat diet was significantly suppressed. Catechins and mRNA expressions of acyl-con oxidase (ACO) and medium chain acyl-con dehydrogenase (MCAD), which are related to B-oxidation within the liver were upregulated. It was also demonstrated that weight gain, body fat, accumulation, TG and TC in the liver, and TG in serum were suppressed in rats fed on normal diet and water containing 0.5% catechins. Additionally, a diet containing 1% catechins decreased TG levels and enzymatic activities of fatty acid synthesis (F & S) on enzymes involved in fatty acid synthesis in rat liver. Rats fed with a

high-fat diet with 0.05% or 0.14% caffeine demonstrated a reduction in body fat mass as well as hepatic and serum TC and TG levels. Moreover, serum catecholamine concentrations were increased in rat that were orally administered 5mg/kg of caffeine.

Taxonomy

Class: Equisetopsida

Subclass: Magnolidae

Superorder: Rosanae

Order: Malvales

Family: Malvaceae

Genus: cola

Common name (s): Kola nut, cola, kola, siffer kola (English)

Abata cola, Gbaja cola, goro cola, labozlic kola (traditional names).

2.3 GEOGRAPHY & DISTRIBUTION

Cola nitida is native to West Africa (from Guinea to Ghana) and has been introduced throughout the forested areas of West and Central Africa. Commercial crops are grown mainly in Nigeria,

Ghana, Cote d'ivoire and Sierra Leone and also to some extent in India, Brazil and Jamaica.

2.4 LIPOPROTEIN

A lipoprotein is a biochemical assembly that contains both proteins and lipids. Many enzymes, transporters, antigens, adhesions, structural proteins and toxins are lipoproteins. The trans membrane proteins of the mitochondrion and the chloroplast, and bacterial lipoprotein functions of lipoprotein particles is to transport water insoluble lipids (fats) and cholesterol around the body in the blood. The various types of lipoprotein in the body enable fats to be carried in the blood stream. The lipoproteins have hydrophilic groups of phospholipids, cholesterol and apoproteins directed outward. Such characteristics make them soluble in the salt water based blood pool. Triglyceride – fats and cholesterol esters are carried internally, shielded from the water by the phospholipids monolayer and the apoprotein.

The interaction of the proteins form the surface of the particles with :

- a. Enzymes in the blood
- b. Specific proteins on the surfaces of cells which determine whether triglycerides and cholesterol will be added to or removed from the lipoprotein transport particles.

TYPES OF LIPOPROTEIN

Cholesterol is carried in the blood by molecules called lipoproteins. A Lipoprotein is any complex or compound that contains both lipid (fat) and protein. The three main types are.

-HDL (high density lipoprotein) : People refer to it as a good cholesterol. HDL does the opposite of LDL – HDL takes the cholesterol away from the cells and back to the liver. In the liver it is either broken down or expelled from the body as waste. Experts say HDL prevents arterial disease.

-LDL (low density lipoprotein) : It is often referred to as a bad cholesterol. LDL carries cholesterol from the liver to cells. There can be a harmful buildup of LDL when the concentration is high. This lipoprotein can increase the risk of arterial diseases and

atherosclerosis if levels rise too high. Most human blood contains approximately 70% LDL – this may vary, depending on the person.

-Triglycerides – this is the chemical form in which most fat exists in the body, as well as in food. They are present in blood plasma. The plasma lipids (blood fat). Triglycerides in plasma originate either from fats in our food, or are made in the body from energy sources, such as carbohydrate calories we consume, but are not used immediately by our tissues. These consumed carbohydrates are converted into triglycerides and stored in fat cells. When your body needs energy and there is no food to serve as an energy source, triglycerides will be released from fat cells and used as energy. Hormones control this process.

FUNCTIONS OF CHOLESTEROL

- It converts sunshine to vitamin D. We were surprised to find that taking vitamin D supplements does not seem to reduce the risk of cholesterol-related cardiovascular diseases.
- It is important for the metabolism of fat soluble vitamins, including vitamins A, D, E, and K.

- It insulates nerve fibers
- It prevents crystallization of hydrocarbons in the membrane.
- It is involved in the production of sex hormones (androgens and estrogens).

CAUSES OF CHOLESTEROL

- Bodyweight – people who are overweight/obese are much more likely to have higher LDL levels and lower HDL levels, compared to people who are of normal weight.
- Smoking – this can have quite a considerable effect on LDL levels.
- Nutrition – although some foods contain cholesterol, such as eggs, kidneys, and some seafood, dietary cholesterol does not have much of an impact in human blood cholesterol levels. However, saturated fats do. Foods high in saturated fats include red meat, some pies, sausages, hard cheese, lard, pastry, cakes, most biscuits, and cream (there are many)
- Alcohol – people who consume too much alcohol regularly, generally have much higher levels of LDL and much lower levels of

HDL, compared to people who abstain or those who drink in moderation.

2.5 HIGH CHOLESTEROL DIAGNOSIS

Cholesterol levels may be measured by means of a simple blood test. It is important not to eat anything for at least 12 hours before the blood sample is taken. The blood sample can be obtained with syringes, or just by pricking the patient's finger. The blood sample will be tested for LDL and HDL levels, as well as blood triglyceride levels. The units are measured in mg/dl (milligrams/deciliter) or 5mmol/liter (millimoles/liter)

Treatment for high cholesterol

- 1) Regular physical activity
- 2) Healthy eating

2.6 LOW CHOLESTEROL

People who have risks of liver disease should strive to keep their cholesterol levels down. However, below 5mmol/l is even more

controversial. Some researchers argue that because cholesterol-lowering drugs mostly appear to have minimal side-effects, almost everyone should take them, but other experts argue that the evidence does not show any particular benefit for certain low-risk groups such as woman who do not have a history of liver diseases, and some point to recent concerns about side-effects, such as damage to muscles, heart or the kidneys.

Very low density Lipoprotein (VLDL) is a type of lipoprotein made by the liver. VLDL is one of the five major groups of Lipoproteins (chylomicrons, VLDL, Low-density lipoprotein intermediate-density lipoprotein, high – density lipoprotein) that enable fats and cholesterol to move within the water-based solution of the bloodstream. VLDL is assembled in the liver from triglycerides, cholesterol, and apolipoprotein. VLDL is converted in the bloodstream to low-density lipoprotein (LDL). VLDL particularly has a diameter of 30 – 80nm. VLDL transports endogenous products, whereas chylomicrons transport exogenous (dietary) products; VLDL transports endogenous triglyceride, phospholipids, cholesterol, and

cholesteryl esters. It functions as the body's internal transport mechanism for lipids.

Treatable Medical Conditions

These medical conditions are known to cause LDL to rise. They are conditions which can be controlled medically (with the help of you, doctor, they do not need to be contributory factors):

- Diabetes
- High blood pressure (hypertension)
- High levels of triglycerides
- Kidney diseases
- Liver diseases
- Under-active thyroid gland.

Risk factors which can not be treated

- Your sex – men have a greater chance of having high blood cholesterol level than women
- Your age – as one gets older, one's chances of developing atherosclerosis increases.

- Early menopause – women whose menopause occurs early are more susceptible to higher cholesterol levels, compared to other women.

2.7 CHANGES DURING CIRCULATION

Nascent VLDL released from the liver contains apolipoprotein B-100, apolipoprotein C1 (apoC1), apolipoprotein E (apoE), cholesterol, cholesteryl esters, and triglycerides. As it circulates in blood, it picks up apolipoprotein C-11 (apoC-11) and an additional apoE donated from high-density lipoprotein (HDL). At this point, nascent VLDL becomes a mature VLDL. Once circulation occurs, VLDL will come in contact with lipoprotein lipase (LPL) in the capillary beds in the body (adipose, cardiac, and skeletal muscle). LPL will remove triglycerides from VLDL for storage or energy production. VLDL now meets back up with HDL where apo C- 11 is transferred back to HDL (but keeps apo E). HDL also transfers cholesteryl esters to the VLDL in exchange of phospholipids and triglycerides via cholesteryl ester transfer protein (CETP). As more

triglycerides are removed from the VLDL because of the action of LPL and CETP enzymes, the composition of the molecule changes and it becomes an intermediate – density lipoprotein (IDL).

Fifty percent of IDLs are recognized by receptors in the liver cells because of the apolipoprotein B-100 (apo B-100) and apo E they contain and are endocytosed.

2.8 LIVER

The Liver is the largest organ inside the body. In adults, it is about the size of a football and weighs close to three pounds. It is located behind the ribs in the upper right-hand portion of the abdomen. Shaped like a triangle, the liver is dark reddish-brown and consists of two main lobes. As said earlier, there are over 300 billion specialized cells in the liver that are connected by a well organized system of “bile” ducts and blood vessels called the biliary system. The liver is such an important organ that we can survive only one or two days if it shuts down -- if the liver fails, the body will fail, too. Fortunately, they can function even when up to 75% of it is diseased

or removed. This is because it has the amazing ability to create new liver tissues (it can regenerate itself) from healthy liver cells that still exist. If the body is an automobile, the liver would be considered the engine; it does hundreds of vital things to make sure everything runs smoothly in the body system.

The important functions of the liver include

- Stores vitamins, sugar and iron to help give the body energy.
- Controls the production and removal of cholesterol.
- Clears the body of waste products, drugs, and other poisonous substance.
- Makes clotting factors to stop excessive bleeding after cuts or injuries.
- Produces immune factors and removes bacteria from the bloodstream to combat infections.
- Releases a substance called “bile” to help digest food and absorb important nutrients.

Hepatitis B

Hepatitis B is the most common serious liver infection in the world. It is caused by the hepatitis B virus (H B V), which attacks liver cells

and can lead to cirrhosis. The virus can be transmitted through contact with infected blood and bodily fluids that contain the blood.

Approximately 10% of adults, 30-50% of children, and 90% of babies who do not get rid of the virus will develop a chronic H B V infection. Chronically infected people can pass the virus on to others and are at increased risk of liver problems later in life, hepatitis B can be prevented with a safe and effective vaccine.

CHAPTER THREE

MATERIALS AND METHODS

3.1 IDENTIFICATION AND EXTRACTION OF PLANT MATERIAL

Healthy green-coloured cola nitida pods were bought from Ogbete main market, Enugu State in the month of November 2012. The pods were identified and authenticated by a plant taxonomist: Prof Okafor of the Biotechnology Department, University of Nigeria, Nsukka.

The cola pods were dried at a room temperature for eighteen (18) days. The pods were later grounded into fine powder with the aid of a clean dry electric grinder (Moulinex, optiblend 2000, made in France). A 130g portion of the ground pod was soaked in 130ml of distilled water for twelve (12) hours, filtered and then extracted with double distilled water by hot-continuous percolation method in a Soxhlet apparatus. The water solvent in the extract was then distilled off in a distillatory and evaporated to dryness at 40⁰C.

The solid extract weighing 18.3g was placed in a sterile container labeled and stored at 40°C in a refrigerator. The 18.3g was later divided into two containers (6.3g and 12g). The first container of 6.3g was used for experimental animal model while the other of 12g was used for phytochemical analysis.

3.2 PHYTOCHEMICAL ANALYSIS

The phytochemical test on the solid extract was carried out based on procedures outlined by Harbourne (1973) and later modified by Trease and Evans (1996).

The phytochemical tests done and their procedures are:

1) Steroid Determination

- Weigh 1g of sample
- Macerate with 20mls of ethanol
- Filter
- Pipette 2mls of the filtrate
- Add 2mls of colour reagent
- Stand for 30 minutes

- Measure absorbance at 550nm

2) Saponin Determination

- Weigh 1g of sample
- Macerate with 10mls of petroleum ether
- Decant into a beaker
- Add another 10mls of petroleum ether
- Decant into the beaker
- Combine the filtrate
- Evaporate to dryness
- Add 6mls of water extract pod
- Pipette 2mls into a test tube
- Add 2mls of colour reagent
- Stand for 30 minutes
- Measure absorbance at 550nm

3) Flavonoid Determination

- Weigh 1g of sample
- Macerate with 20mls of ethyl acetate
- Filter

- Pipette 5mls of the filtrate
- Add 5mls of dilute ammonia slake
- Collect the upper layer and measure absorbance at 490nM

4) Reducing Sugar Determination

- Weigh 1g of the sample
- Macerate with 20mls of distilled water
- Pipette 1ml of the filtrate; add 1ml of alkaline copper reagent
- Boil for 5 minutes, cool
- Add 1ml of phosphomolybdic acid reagent
- Add 7mls of distilled water
- Measure absorbance at 420nM

5) Alkaloid Determination

- Weigh 1g of the sample
- Macerate with 20mls of 20% H_2SO_4 in ethanol (1:1)
- Filter
- Pipette 1ml of the filtrate
- Add 5mls of 60% H_2SO_4 and 5mls of 0.5% formaldehyde in 60% H_2SO_4

- Mix
- Allow to stand for 3 hours
- Measure absorbance at 565nm

6) Terpenoid Determination

- Weigh 1g of the sample
- Macerate with 50mls of distilled water
- Filter
- Pipette 2.5mls of the filtrate
- Add 2.5mls of 5% aqueous phosphomolybdic acid solution
- Add 2.5mls conc H₂SO₄ gradually
- Mix
- Allow to stand for 30 minutes
- Make up to 12.5mls with ethanol
- Measure absorbance at 700nm

7) Glycoside Determination

- Weigh 1g of the sample
- Add 2.5ml of 15% lead acetate
- Filter

- Add 2.5mls of chloroform
- Shake vigorously
- Collect the lower layer and evaporate to dryness
- Add 3mls of glacial acetic acid
- Add 0.1ml of 5% ferric chloride and 0.25ml conc. H_2SO_4
- Shake
- Put in the dark for 2hours
- Measure absorbance at 530nm

8) Tannin Determination

- Weigh 1g of the sample
- Macerate with 50mls of methanol
- Filter
- Pipette 5mls of the filtrate
- Add 0.3mls of 0.1M ferric chloride in 0.1M HCl
- Add 0.3mls of 0.0005M potassium ferricyanide
- Measure absorbance at 720nm

9) Cyanide Determination

- Weigh 1g of the sample

- Macerate with 50mls of distilled water
- Stand for 24hrs
- Filter
- Pipette 1ml of the filtrate
- Add 4mls of alkaline picrate solution
- Boil for 5 minutes
- Cool
- Measures absorbance at 490nm

10) Soluble Carbohydrate Determination

- Weigh 1g of the sample
- Macerate with 50mls of distilled water
- Filter
- Pipette 1ml of the filtrate
- Add 2mls of saturated picric acid
- Measure absorbance at 530nm

3.3 EXPERIMENTAL ANIMAL MODEL

Twelve (12) healthy male albino wistar rats with mean weight of 1.50 ± 0.60 kg were obtained locally from Nsukka, Enugu State. The rats were randomly distributed into four (4) groups (I-IV) of three (3) rats each. They were housed separately and fed with water and grower's mash (Guinea feed Nigeria) and allowed for 3 days to acclimatize. A high lipoprotein food (cow's brain) was prepared by dissolving 300g of fresh cow's brain in 500ml of distilled water forming a semi-solid mixture. A known antilipidemic drug; Atovarstatin (brand name-Lipitor, 10mg) was prepared by dissolving 5mg (half of one tablet) in 2ml of distilled water. Also cola pod nitida water extract weighing 6.3g was dissolved in 17ml of distilled water forming a liquid drug extract. Group I rats were fed orally with 6ml of the lipoprotein food mixture which contains 3.6g of the cow's brain twice a day for seven (7) days. Group II rats were fed orally with only grower's mash and water.

Rats in group III were also fed orally with 6ml of the lipoprotein food mixture for seven (7) days and were later administered orally with the

2ml dissolved atorvastatin drug for the following three (3) days. Each group IV rats were fed orally with 6ml of the lipoprotein food mixture for seven (7) days and later were administered orally with 0.5ml of the liquid drug extract (cola pod nitida extract) for the three (3) days.

3.4 COLLECTION OF BLOOD SAMPLES

The collection of blood samples from the rats in each group was simply done by dissecting the rats, followed by cardiac puncture after a mild anaesthesia with chloroform. About 5-9mls of blood samples was collected in an EDTA tube from each group using a medical syringe. Serum was separated from the blood after clotting by centrifugation and then used for lipid analysis.

3.5 LIPID PROFILE ANALYSIS

In the lipid profile analysis, tests are conducted for serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triacylglycerol. Low-density lipoprotein cholesterol (LDL-C) is then calculated using a standard formula.

1) TEST FOR TOTAL CHOLESTEROL (TC)

(a) Material/reagents involved

- Serum
- Ferric chloride reagent (2.5g of ferric chloride mixed with 100ml of 85% phosphoric acid)
- Standard cholesterol (250mg of pure cholesterol mixed with 100ml of glacial acetic acid)

(b) Procedure

- Dilute the serum at about 1:20 with distilled water
- Dilute the standard cholesterol at about 1:20 with glacial acetic acid
- Using three glass test tubes labelled – Test, Standard and Blank respectively, the following procedure was conducted

Sample	Test	Standard	Blank
Ferric chloride reagent	5.0ml	5.0ml	5.0ml
Diluted serum	0.5ml	-	-
Diluted standard cholesterol	-	0.5ml	-
Distilled water	-	-	0.5ml

Shake the tubes very well for about 10 seconds in order to mix-up the contents.

Immediately drop the test tube in a boiling water bath for about 90 seconds.

Cool for about 5 minutes with running tap water.

Read the absorbance at 560nm against the blank with a dry cuvette.

Finally, calculate using the formular:

$$\frac{\text{Absorbance test} \times 250 \text{ (constant value)}}{\text{Absorbance standard}}$$

The unit is mg/dl

2) TEST FOR HDL-CHOLESTEROL (HDL-C)

(a) Materials/Reagents involved

- Serum
- Cholesterol standard (100mg of pure cholesterol mixed with 100ml of glacial acetic acid)
- Colour reagent (5.6g of 2,5 – dimethyl benzene sulphuric acid mixed with 200ml of glacial acetic acid and 300ml of acetic anhydride)
- Phosphotungstic acid reagent (4.5g of phosphotungstic acid mixed with 50ml of water, add 16ml of 1N NaOH and make up to 100ml with water)

- Tris buffer (1.21g of tris mixed with 90ml of water, then the pH was reduced to about 7.6 with 1N HCL and diluted up to 100ml with water)

(b) Procedure

- Pipette about 1ml of serum in a test tube.
- Drop 0.1ml of phosphotungstic acid reagent and mix very well
- Add 0.05ml of magnesium chloride and mix very well
- Centrifuge at 2500rpm or 1500g for 30 minutes
- Carefully remove the clear supernatant with a Pasteur pipette
- Add 2 drops of the colour reagent and allow to stable for 15 minutes
- Read the absorbance at 560nm

3) TEST FOR LDL-CHOLESTEROL (LDL-C)

The LDL-Cholesterol is calculated using the standard formula: Total cholesterol-HDL-Cholesterol + 0.46 (constant value) .

4) TEST FOR TRIGLYCERIDES

(a) Material/Reagents Involved

- Serum
- Heptane

- Isopropanol
- Sodium methylate (50mg of sodium methylate diluted in 100ml of isopropanol)
- Sulphuric acid (0.08N) – Prepared by mixing 2.2ml conc H₂SO₄ of about 36.0N with 500ml of distilled water. The dilution should be up to 1 litre.
- Periodate reagent (1.23g) of N₁O₄ was mixed with 100ml of 0.88N, about 5% v/v acetic acid. Then stored in a brown bottle)
- Acetylacetone reagent (0.75ml of acetyl acetone was dissolved with 2.5ml of isopropanol, then 2N of ammonium acetate (15.4%) was added to make the volume up to 100ml then stored in a brown at 4°C
- Triglyceride standard (200mg of pure triolein was mixed with 100ml of isopropanol)

b) Procedure

- Using three glass test tubes labelled – Test, Standard and Blank respectively, the following procedures was conducted

Sample	Test	Standard	Blank
Serum	0.5ml	-	-
Triglyceride	-	0.5ml	-

standard			
Distilled water	-	0.5ml	0.5ml
Isopropanol	3.5ml	3.0ml	3.5ml
H ₂ SO ₄ (0.08N)	1.0ml	1.0ml	1.0ml
Heptane	2.0ml	2.0ml	2.0ml

Shake the tubes for 30 seconds to mix very well. The tube should stand for 10 minutes at room temperature for proper separation of two layers. Prepare another set of three test tubes labelled – Test, standard and Blank respectively and the following procedure was done.

Sample	Test	Standard	Blank
Top solvent layer from respective tubes	0.2ml	0.2ml	0.2ml
Sodium methylate	3.0ml	3.0ml	3.0ml

-Shake the tubes using vortex mixer to mix very well

-Incubate at 60°C for 10 minutes and then cool at room temperature

-Colour development stage, which involves dissolving 0.1ml of periodate reagent to each tube and mix very well. Then cool at room temperature after 10 minutes of incubation at 60°C

-Centrifuge the tubes and transfer the upper phase liquid to another fresh tube

-Using spectrophotometer, read the absorbance of test and standard at

N420nm against the blank

-Finally calculation is done by

$$\text{Triglyceride (mg/dl)} = \frac{\text{Absorbance of Test} \times 200}{\text{Absorbance of Standard}}$$

Then to convert to mmol/dl is $\text{mg/dl} \times 0.0113$ (standard value)

CHAPTER FOUR

PHYTOCHEMICAL COMPOSITION OF SAMPLES

Sample code	Solvable CHO	Cyanide	Reducing sugar	Saponin	Tannin	Flavonoid	Actalorid	Steroid	Terpenoids
A	1.43± a 0.004	0.64±a 0.003	326.090a a±0.005	1.45±a 0.002	2.64±a 0.0105	3.25±a 0.003	3.14±a 0.003	0.09±a 0.004	0.37±a 0.002
B	1.91±b 0.001	0.56±b 0.003	360.806b ±0.004	1.22±b 0.004	2.56±b 0.003	3.92±b 0.003	3.16±b 0.008	0.04±b 0.003	0.45±b 0.004
C	1.43±a 0.005	0.63±a 0.003	321.743c ±0.003	1.05±c 0.003	2.51±c 0.001	3.82±a 0.003	3.18±c 0.039	0.63±c 0.004	0.23±c 0.005
D	1.92±b 0.003	0.52±d 0.003	334.243d ±0.003	1.34±d 0.004	2.14±d 0.003	2.91±d 0.003	3.45±d 0.006	0.51±d 0.004	0.37±d 0.002
E	1.49±c 0.003	0.58±b 0.020	373.652e ±0.006	1.43±e 0.004	3.31±b 0.007	3.35±a 0.004	3.25±e 0.003	0.55 0.007	0.39±e 0.003
F	1.66±d 0.003	0.48±c 0.006	360.664 ± 0.003	1.10± f 0.005	3.30±d 0.004	3.52±a 0.005	3.92±f 0.003	0.62±c 0.004	0.38±e 0.003
G	1.59±e 0.005	0.49±c 0.003	326.4851 ±0.004	1.16±g 0.004	2.51±b 0.004	3.61±a 0.004	3.61±c 0.004	0.66±a 0.095	0.31±f 0.004
H	1.59±f 0.003	0.67±e 0.007	304.357g ±0.005	1.19±h 0.004	2.62±a 0.004			0.65± 0.002	0.34±g 0.002

Data of triplicate determination + Standard Deviation (SD)

Data in the same column bearing different superscript differed significantly (PSO.05).

Key

- A = N-hexane extract of leaf
- B = Ethanol extract of leaf
- C = Water extract of leaf
- D = Ethanol extract of leaf
- E = N-hexane extract of stem
- G = N-hexane extract of pod
- H = Water extract of stem

Qualitative analysis table 2: Qualitative phyiochmistry

	A	B	C	D	E	F	G	H
Tannin	++	+++	+++	+++	+++	+++	+++	+++
Alkaloid	++	+++	+++	+++	+++	+++	+++	+++
Carbohydrate	+	++	+	++	+	+	+	+
Saponin	+	+	+	+	+	+	+	+
Steroid	+	+	+	+	+	+	+	+
Hydrogen	+	+	+	+	+	+	+	+
Cyanide								
Flavonoid	++	++	++	++	++	++	++	++
Reducing Sugar	++	++	++	++	++	++	++	++
Terpenoid	++	+	+	+	+	+	+	+

Key

- A = N – hexane extract of leaf
- B = Ethanol extract of leaf
- C = Water extract of leaf
- D = Ethanol extract of leaf
- E = N – hexane extract of stem
- G = N – hexane extract of pod
- H = Water extract of stem

TABLE 3

Rats	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglyceride (mg/d)
Normal feed (G P ₁)	140.00 \pm 1.41a	30.00 \pm 1.41a	3.60 \pm 0.19	95.00 \pm 1.44a
Cow's brain (GP ₂)	145.00 \pm 1.41b	40.00 \pm 1.41b	3.90 \pm 00b	105.00 \pm 1.41
Lipitor (GP ₃)	110.00 \pm 0.00c	13.00 \pm 1.41c	1.20 \pm 0.14e	39.00 \pm 1.41c
Pod water extract (GP ₄)	140.00 \pm 1.41	35.00 \pm 1.49 ¹	2.08 \pm 0.00	55.00 \pm 0.00d

Data are meaning of duplicate determination \pm standard deviation (SD) Data in the same column carrying different superscript are significantly different (P<0.05).

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The Kola pod nitida contains many species numbering up to 50 in West Africa, the major kola are known as *C. Nitida* which is known as Gbanja or Goro among the Hausa tribe in Nigeria. *Cola nitida* and *cola acuminata* are of major economic and social importance in Nigeria. (Russelu 2005).

The observation in this study supports that kola pod husk shares several similarities with coco pod husk. As said earlier, both have high crude protein and low crude fibre content. *Kola nitida* pod husk contain the highest value of calcium (0.60%) chloride ion (0.44%) and molybdenum (9.4ppm) (lowest value of this nutrient is observed in kola test) (0.7ppm). The seeds of both *cola nitida* and *cola accuminata*, its nutritive quality have been reported. In the lipid profile analysis, tests were conducted for serum total cholesterol (TC), high density lipoprotein cholesterol (HDL – C) and triacylglycerol.

Low – density lipoprotein cholesterol (LDL – C) was then calculated using a standard formula.

5.2 CONCLUSION

It can be said that, cola nitida increases gastric acid secretion in wistar rats. The indication of gastric acid secretion of cola nitida could be entirely due to the presence of xanthin or it may involve gastric secretagogues in cola yet to be discovered. Tests have to be done in order to determine if they are potable in pod.

REFERENCES

- Abloye, J., Fanimu, A. O., Bamgbose, A. M., Dipeolu, M. A., & Olubamiwa, O. (2006). Nutrient utilization, growth and carcass performance of broiler chickens fed graded levels of kolanut husk. Nigeria. *J Poult Sci.* **43**: 365-370.
- Adaramoye, A. O. (2010). *Protective effect of Kolaviron, a biflavonoid from Garcinia kola seeds in brain of Wistar albino rats exposed to gamma radiation.* Nigeria: Biol Pharm Bull.
- Amos, A. F., Mc-Carty, D. J., & Zimmet, P. (1997). *The Rising Global Burden of Diabetes and its complications: Estimates and projections to the year 2010.* England : Diab Med. **14**: 551-585.
- Babatunde, B. B., Hamzate, R. A., & Adejinmi, O. O. (2001). *Replacement Value of Kolanut husk meal for maize in rabbit diet.* Nigeria : Trop J An Im Sci. 4(2): 127-133.
- Babatunde, B. B., Hamzat, R. A. (2005). *Russellu effects of feeding graded levels of cockerels.* Nigeria: Longman Publishers.
- Beckstrom, S. M., Sternberg, J. A., Duke, I. (1994). *The Phytochemical database.* U.S.A: Arsgenome Cornell Edu.
- Blades. M. (2000). *Functional Foods or Neutral Central Nutrition and food science.* USA : Prentice Hall Inc.
- Burtis, C. A., & Ashwood, E. R. (1986). *Textbook for clinical chemistry.* Philadelphia, Pennsylvania: W.B. Saunders Company.
- Esimone, C. O., Adikwu, C. S., Okoye, C., & Odimegwu, D. C. (2007). *Adaptogenic potentials of camellia sinensis leaves, cola nitida and Garcinia kola.* Nigeria: Longman Publishers.

- Haustein, A. (1971). *Colanut: Custom and Rituals*. Sudan: Anthropos Publishers.
- Jensen, G. E., Stainberg, S. E., Freese, P., & Marino, E. (1996). Liver function tests. *J. Digest Disord*, 2004, 6:1-3.
- Kapil, O., Suri, P., & Koul, I. B. (1995). *Antihepatotoxic effects of chorogenic acid from Authrocephalus cadamba phytother*. India: Mumbai Publishers.
- Kothavade, R. J., Joylekar, S. N., & Barodavalla, S. A. (1996). Protective effect of indigenous drug livomyn on ketoconazole induced hepatotoxicity. *Indian J. Pharm. Sci.*, 58:142-146.
- Lim, K. T. (2012). *Edible medicinal and non-medicinal plants*. London: Springer Press.
- Mabberley, D. J. (2008). *Mabberley's plant-book: a portable Dictionary of Plants, their classification and uses*, 3rd Edition. New York: Cambridge University Press.
- Plaa, G., & Charbonneau, M. (1982). *Detection and Evaluation of Chemically induced liver injury*. France: Frito Publishers.
- Vaughan, J. G., & Geissler, C. A. (2009). *The New Oxford Book of Food Plants*, 2nd Edition. New York: Oxford University Press Inc.
- Williamson, D. T., Okpako, E. M., & Evans, F. J. (1996). *Selection, preparation and pharmacological Evaluation of plant material*. England: John Wiley and Sons.
- Widjaja, M. (2012). *Insight into Igbo Culture, Igbo Language and Enugu* Nigeria: Longman Publishers.

Zhout, L. D., & Lix, L. (2009). Hypoglycemic and hypolipidemic effects of flavonoids from lotus (*Nelumbo nocifera* G. aertn) leaf in diabetic mice. *J. med. Plant Res.* 3 (4):290-293.