

TITLE PAGE

**ANTILIPIDEMIC EFFECT OF WATER (H₂O) EXTRACT OF
DESMODIUM VELUTINUM LEAVES ON ALBINO WISTAR RATS**

BY

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CERTIFICATION

This is to certify that this report titled antilipidemic effect of water extracts of *Desmodium velutinum* leaves on albino wistar rats submitted by Barratt Mary Eni BC/2009/282 is a borne-fide record of the project work carried out by her under my supervision.

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(Supervisor)

Date.....

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Mr. Ezenwali Moses
Head of Department

Date.....

.....
External Supervisor

Date:

DEDICATION

This project work is dedicated to the TRINITY “GOD THE FATHER, GOD THE SON AND GOD THE HOLY SPIRIT”, for their divine favour.

ACKNOWLEDGMENT

My special thanks goes to the almighty God for his grace and provision to finish this project.

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ABSTRACT

This study evaluated the antilipidemic activity of water extracts from leaves of *Desmodium velutinum* on albino wistar rats. The phytochemical analysis of the leaf extract showed the presence of tannins, saponins, alkaloids, soluble carbohydrates, flavonoids, reducing sugar, steroids, cyanide and terpenoids. The animals were treated with known drugs (atorvastatin 2ml). There were significantly reductions in HDL 13.00 ± 1.41 mg/dl LDL 1.20 ± 0.14 mg/dl and triglyceride 39.00 ± 0.14 mg/dl, compared with water extract of *Desmodium velutinum* (0.5ml). when administered was found to significantly reduce lipid plasma which was LDL 1.90 ± 0.00 mg/dl, triglyceride 50.00 ± 0.00 mg/dl and increase HDL 25.00 ± 0.00 mg/dl which is the good cholesterol. The water extract of *Desmodium velutinum* leaf can possibly normalize the plasma lipid when compared with the group given atorvastatin. The phytochemicals analysis showed that the association between these complexes and compounds and other constituent play an important role in the biological activity of the leaf. This study suggested that the water extract of *D.velutinum* leaf posses hypolipidemic as well as antilipidemic effect.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Lipid and lipoprotein abnormalities play a major role in the development and progression of coronary artery diseases. Low levels of high density lipoprotein cholesterol have been identified as independent coronary risk factors (Rodrigue *et al.*, 2010). High level of blood cholesterol is responsible for circulatory system disorder. Increase level of low density lipoprotein (LDL) is alarming for cardiovascular diseases and their risk is increased many times (Harman *et al.*, 2011).

In developing countries, the occurrence of heart diseases increases rapidly (Nordestgard *et al.*, 2010). Medical studies show that about 70% of adults over 50 years old suffer atherosclerosis. (Sherien and Azza, 2009). A large number of synthetic hypolipidemic drugs are available in market. Long term use of these drugs cause serious side effects, and are costly.

A medicinal plant is any plant which in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs (Sivakumar *et al.*, 2007). Plant contains a large number of bioactive phytochemicals that are responsible for pharmacological action of plants and used for development of drugs. Many

medicinal plants have shown their antilipidemic effect and proved their efficacy in cardiovascular diseases (Nordestgard *et al.*, 2010; Wang, 1999).

One of such plants used very often in the management of the disease by the traditional medicine practitioners of Eastern Nigeria is *Desmodium velutium*, a perennial plant erect or semi-erect shrub or sub-shrub up to 3m light. It is widely distributed in subtropical Asia and tropical Africa. (Amowi and Azode, 2012).

Extracts of *Desmodium velutium* are used traditionally in some disease conditions particularly aphrodisiac and headache. Hence, *Desomdium velutium* may be a source of a pharmacological active agent useful in the treatment of aches, pains and diarrhoea. In Ghana, native doctors mix the root of *Desmodium lasincarpum* with some hot peppers and use it as enema to cure blood in urine. In Eastern States of Nigeria, the plant locally known as “Ikeagwuani”. (Onyegbule *et al.*, 2012). In these present studies, I investigated the antilipidemic activity of the water (H₂O) extract of *Desmodium velutinum* Leaves on albino wistar rats.

CHAPTER TWO

2.0 LITEFATURE REVIEW

2.1 *DESMODIUM VELUTIMUM*

Desmodium velutinum is an upright woody perennial herb, sub-shrub or shrub, up to 3m tall (Onyegbule, *et al.*, 2012).

Leaflets are light green to blue, green or darker above, paler beneath and with prominent pallid veins, roundish to elliptic to rhombic ovate, 3-9.4cm long , 2-7cm wide, entire or repaid (indented at the termination of the lateral veins) and ciliate, thick, mostly soft velvety on both Surface with long stiff golden, reddish or white hairs. Inflorescence arises in leaf axils, and at the end of branches. They are densely flowered racemes up to 10cm or more long; at least the terminal ones often paniculately branched. Flowers are white pink to blue, mauve or brilliant purple, usually appearing whitish or pale when dry.

Common names – velvet leaf D, villous LD

Hind: *Jagru, Lagavang, Lippa-Pank*

Malayalam: *Orial, Sanskrit, Prasnipani*

Tamil: *Akilametaki, Amcapatayilni, Ankachupati*

Telugu: *Cluia madu, chimanduri*

India: *Chilkiboota*

India: *Lalkan* while local name: *Ikeagwuani* (Igbos)

Botanical Name: *Desmodium velutinum*

Family: *Fabaceae* (Pea family)

Order: *Fabales* **Class:** *Magnoliopsida*, **Division:** *Magnoliophyta*, **Genus:** *Desmodium* (Garg, 2006)

Other species of *Desmodium* include *Desmodium lasiocarpum*, *Desmodium gangeticum*, *Desmodium repandum*, *Desmodium styracitolum*, *Desmodium diffusum*, *Desmodium heterophyllum*, *Desmodium triflorum*, etc.

Desmodium velutinum can adapt to a wide range of soil PH, from very acid (pH 4.0) to alkaline. It also prefers more humidity climates of >1000>3000mm rainfall 1 year, tolerates up to 5 months dry season. It grows at altitudes from 0.1-500m with average temperature above 20⁰C and has some shade tolerances as it grows in forest verges in New Guinea.

2.1.1 BIOLOGICAL AND MEDICINAL USE OF DESMODIUM VELUTINUM

The water extract of *Desmodium velutinum* is use as an aphrodisiac (a substance that increases sexual desire, example of such plants includes tamera aphrodisiac, zingibar Offieinale, *Mucuna Pruriens* etc).

In South-East Asia, they are considered other prominent uses are the treatment of diarrhea, dysentery and stomach ache. *Desmodium velutinum*

may be a source of pharmacological active agent useful in the treatment of aches, pains and pyretic. It is also have diuretic effects (a substance that causes an increase in the flow of urine)

2.1.2 USES OF OTHER SPECIES OF *DESMODIUM*:

Desmodium shows a wide range of medicinal uses. In Ghana, native doctors mix the roots of *Desmodium lasiocarpum* with some hot-peppers and use it as enema to cure blood in urine. In the Philippines, a decoction of *Desmodium triflorum* is used as a mouth wash and as a expectorant (a cough medicine that helps you to get rid of thick liquid from the lungs). In India, fresh leaves of *Desmodium triflorum* are used internally as a *galactagogue* (a substance that increases lactation milk supply), some of the most commonly used herbal *galactagogues* are fenugreek, blessed thistle, alfalfa; and in Taiwan, the whole plant is used against fever, rheumatism, Jaundice and gonorrhoea. *Desmodium incanum* is used as a diuretic, stomachic, Febrifuge and hemostatic in Central America. *Desmodium heterocarpon* are primarily forages, but are also used medicinally in Malesia. The boiled roots of *Desmodium heterocarpon* are used in Malaysia to poultice sore breasts, and a decoction of the plant is regarded as a tonic and a bechic (a cough suppressant). In Cambodia, the stems of *Desmodium heterocarpon* are

applied to fracture and snake bite. In Taiwan, a decoction of the root is used against rickets in children.

Desmodium heterophyllum is applied in Malaysia to treat sores, earache, stomach-ache and abdominal complaints. In India, the roots are considered carminative (a herb that either prevents formation of gas in the gastrointestinal tract or facilitates the expulsion of gas, thereby combating flatulence), tonic and diuretic, the leaves are used as a *galactagogue* and a decoction of the whole part plant is used to treat stomach-ache and abdominal problems. *Desmodium gangeticum*, *Desmodium sequax*, *Desmodium styracifolium*, *Desmodium repandum* are use in treating wounds, ulcers, toothache, stones in the gall bladder, kidneys or bladder and other skin problems.

2.2 PLANTS FOR ANTILIPIDEMIC

Other plants for lowering lipoprotein level in the body.

***Terminalia Arjuna*:** Botanical name

Terminalia arjuna is a big evergreen tree up to 25meters high, bark grey, smooth leaves, flower small and fruits are 2.3-2.5m long. It has great importance due to its curative properties in heart problems. Phytochemicals belonging to different classes are present in the bark of *terminalia arjuna*

including tannins, triterpenoids, saponina, arjunic acid, arjunolic acid, arjungenin (Manna *et al.*, 2007).

Experimental studies revealed its dark shaved significant antioxidant (Vaidya *et al.*, 2008; Shridhar and Gopal, 2009), antidiabetic (Raghavan and Kumari, 2006) antigastric ulcer (Devi *et al.*, 2007), antimutagenic (Vaidya *et al.*, 2008), anthelmintic, (Bachaya *et al.*, 2009) activities.

The bark is useful in cardiovascular diseases, especially in disturbed cardiac rhythm angina or myocardial infraction. Clinical studies suggested that it improves the blood circulation to heart, regulate blood pressure (Nammi *et al.*, 2003) is used for treatment of hypercholesterolemia (Jiwari *et al.*, 1990; Ram *et al.*, 1997; Chander *et al.*, 2004) and inhibit the platelet aggregation (Namita *et al.*, 2009). It protects liver and kidneys against the harmful effect of free radicals.

Botanical Name- *Trigonella foenum- graecum*.

Trigonella foenum- graecum commonly know as fenugreek (methi) is a widely cultivated aromatic herb varying in height from thirty or sixty centimeter and used both as vegetable (leaves) and spice (seeds) (Toppo *et al.*, 2009). The seeds are known as “Maithray” is used in the preparation of pickles, curry powders. The young leaves are eaten as vegetable and dried

leaves (called Kasuri methi) have a bitter taste and strong characteristic smell.

Experimental studies revealed that *trigonella foenum* seed and leaves extracts possess strong antidiabetic activity (Sharma *et al.*, 2009; Vats *et al.*, 2002). Fenugreek seeds contain high quantity of saponins and galactosamine, whose cholesterol lowering effect is well established (Bahram *et al.*, 2005; Xue *et al.*, 2007).

Cardioprotective and immunomodulatory potential of this important plant is need to be explored.

Botanical Name: *Rheum emodi*

Rheum emodi is commonly known as rhubarb, revand chini. It is a perennial plant that grows from Rhizomes. Rhubarb is an important herb used in ayurvedic medicines. It is a very important hepatoprotective and showed very good results against chemically induced elevated level of AST, ALT and ALP in serum (Ibrahim *et al.*, 2008, Akhtar *et al.*, 2009). Antibacterial (Babu *et al.*, 2003), antifungal (Agarwal *et al.*, 2000), hypoglycemic (Li and Wang, 1997) nephroprotective (Alam *et al.*, 2005), Laxative, appetite stimulant, diuretic and anthelmintic activities.

Cardioprotective and immunomodulatory potential of this plant need to be explored.

Other examples of antilipidemic plants include *coriandrum sativum*, *Euophorbia tirvealli*, *cyperus rotundus* etc.

2.2.1 ADVANTAGE OF MEDICINAL PLANT (ANTILIPIDEMIC PLANTS) OVER SYNTHETIC DRUGS:

Medicinal plant is any plant which in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs. Plants contains a large number of bioactive phytochemicals that are responsible for pharmacological action of plants and used for development of known drugs or a cheap source of known drugs such as reserpine from *ravwolfia* species.

Medicinal plants (Antilipidemic plant) are receiving extra ordinary importance and popularity as safe, efficacious and cost effective medicines with extraordinary benefits due to combination of medicinal ingredients with vitamins and minerals for antilipidemic activity. Many medicinal plants have shown their antilipidemic effects and proved their efficacy in cardiovascular diseases (Jain *et al.*, 2007; Wang, 1999).

Synthetic drugs are those drugs with properties and effects similar to a known hallucinogen or narcotic but having a slightly altered chemical structure, especially such a drug created in order to evade restrictions against illegal substance. In synthetic drugs, the efficacy is not 100% guarantee. The

frequent use of some drugs has severe side effects for example, in atorvastatin (Lipitor) drug, its side effect is diarrhea, headache etc. while *Desmodium velutinum* is use in treating of diarrhea, headache and reduces pain etc. In synthetic drugs, the costly of the latter is increased by modern health technology which in many cases is inappropriate or irrelevant to the immediate needs of people in developing countries.

2.3 LIPIDS AND LIPOPROTEIN:

Definition of Lipid:

Lipid is a broad group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids and others. It is hydrophobic or amphiphilic small molecules.

Definition of Lipoprotein:

Lipoproteins are complex aggregates of lipids and proteins that render the lipids compatible with the aqueous environment of body fluids and enable their transport throughout the body of all vertebrates and insects to tissues where they are required. The proteins serve to emulsify the lipid (otherwise called fat) molecules.

Pathology/Structure of Lipoprotein:

The structure of lipoprotein is similar to plasminogen and TPA (tissue plasminogen activator) and it competes with plasminogen for its binding site, leading to reduced fibrinolysis. Because lipoprotein stimulates secretion of PAL-1, it leads to thrombogenesis. Many enzymes, transporters, structural proteins, antigens, adhesions and toxins are lipoproteins. Examples include the high-density (HDL) and low density (LDL) lipoprotein, which enable fats to be carried in the blood stream, the transmembrane proteins of the mitochondrion and the chloroplast and bacterial lipoproteins.

Lipoproteins are assembled from polar and neutral lipids, as well as specific proteins, which are referred to as apoproteins or apolipoproteins. Apolipoproteins are amphiphilic proteins capable of interacting with both lipids and the surrounding aqueous environment of the plasma. Lipoproteins are synthesized mainly in the liver and intestines. Within the circulation, these aggregates are in a state of constant flux, changing in composition and physical structure as the peripheral tissues take up the various components before the remnants return to the liver.

The most abundant lipid constituents are triacylglycerols, free cholesterol, cholesterol and phospholipids (phosphatidylcholine and sphingomyelin especially), though fat-soluble vitamins and antioxidants are

also transported in this way. Free (unesterified) fatty acids and lyso phosphatidylcholine) are bound to the protein albumin by hydrophobic forces in plasma and in effects are detoxified. The circulating lipoproteins are structurally and metabolically distinct from the proteolipids containing covalently linked fatty acids or other lipid moieties.

The lipoproteins present in plasma are: Chylomicrons (CM), very-low-density lipoprotein (LDL), intermediate-density lipoproteins (IDL) and high-density lipoprotein (HDL).

2.3.1 CLASSIFICATION OF LIPOPROTEINS

Lipoproteins can be classified according to larger and less dense to smaller and denser density. Density is determined largely by the relative concentrations of triacylglycerols and proteins and by the diameters of the broadly spherical particles, which vary from about 6000A in CM to 100A or less in the smallest HDL. Lipoproteins are larger and less dense when the fat to protein ratio is increased. They are also classified on the basis of electrophoresis and ultracentrifugation. It is also possible to classify lipoproteins as “alpha” and “beta” according to the classification of proteins in serum proteins electrophoresis.

Table 1
Major Classes of Human Plasma Lipoproteins: Some Properties
Composition (W %).

Lipoproteins	Density (g/ml)	Proteins	Phospholipids	Free Cholesterol	Cholesterol esters	Triacylglycerols
Chylomicrons	<1.006	2	9	1	3	85
VLDL	0.95-1.006	10	18	7	12	50
LDL	1.006-1.063	23	20	8	37	10
HDL	1.063-1.210	55	24	2	15	4

Principles of Biochemistry Lehninger

Chapter 21, Lipid Biosynthesis Pg. 836.

2.3.2 FUNCTIONS OF LIPOPROTEINS

Lipoprotein and HDL especially play an important role also in host defense as part of the innate immune system. Infection and inflammation induce the acute-phase response, which leads to many changes in lipid and lipoproteins metabolism and initially protects the host from the harmful effects of bacterial, viruses and parasites, produced that the infections are not prolonged.

For example an important defensive function is the ability of HDL and other lipoproteins to binds the endotoxin lipopolysaccharides, which are

primary constituents of the outer membrane of Gram-negative bacteria and so neutralize their toxic effects.

It may function as a multi-ligand binding protein capable of transporting small hydrophobic molecules such as arachidonic acid, steroid hormones and cholesterol for metabolism or signalling. The polar nature of the surface monolayer prevents the lipoprotein particles from aggregating to form larger units.

For example, some are ligands for receptors on cell surfaces and specify the tissues to which the lipid components are delivered, while others are cofactors for lipase or regulate lipid metabolism in the plasma in various ways.

The principal role of the chylomicrons and VLDL is to transport triacylglycerol “forward” as a source of fatty acids from the intestine or liver to the peripheral tissues. In contrast, the HDL removes excess cholesterol from peripheral tissues and delivers it to the liver for excretion in bile in the form of bile acids (reverse cholesterol transport). VLDL transports endogenous triglycerides, phospholipids, cholesterol cholesteryl esters. A function within the coagulation system seems plausible, given the aspects of the high homology between apo (A) and plasminogen. The lipoprotein gene derives from a duplication of the plasminogen gene.

Another possibility, suggested by Linus Pauling, is that lipoprotein is a primate adaptation to L-gulonolactone oxidase (GULO) deficiency, which is found only in certain lines of mammals. GULO is required for converting glucose to ascorbic acid (vitamin C), which is needed to repair arteries, following the loss of GULO, those primates that adopted diets less abundant in vitamin C may have used lipoprotein as an ascorbic acid surrogate to repair arterial walls.

In addition, lipoprotein transports the more atherogenic proinflammatory oxidized phospholipids which attract inflammatory cells to vessel walls with Mac-1 integrin, angiogenesis, wound healings and lead to smooth muscle cell proliferation. Lipoprotein contains a number of important enzymes, including lipases, acyl transferases, transport proteins and some with anti-oxidation and anti-thrombotic effects.

2.4 LIPOPROTEIN DISORDER:

Familial hypertriglyceridemia is a lipoprotein disorder. Its mechanism is by decrease serum triglyceride removal, resulting from decreased lipoprotein lipase (LPL) activity. Increased hepatic secretion of triglyceride-rich VLDL. Its complications, pancreatitis at triglyceride concentrations > 2000mg per deciliter (22.6mmol/liter) low risk of coronary artery disease (CAD).

Familial hypoalphalipoproteinemia is a disorder which is characterized by low concentration of HDL cholesterol and its complication is CAD and peripheral vascular disease (PVD) (may be associated with hypertriglyceridemia).

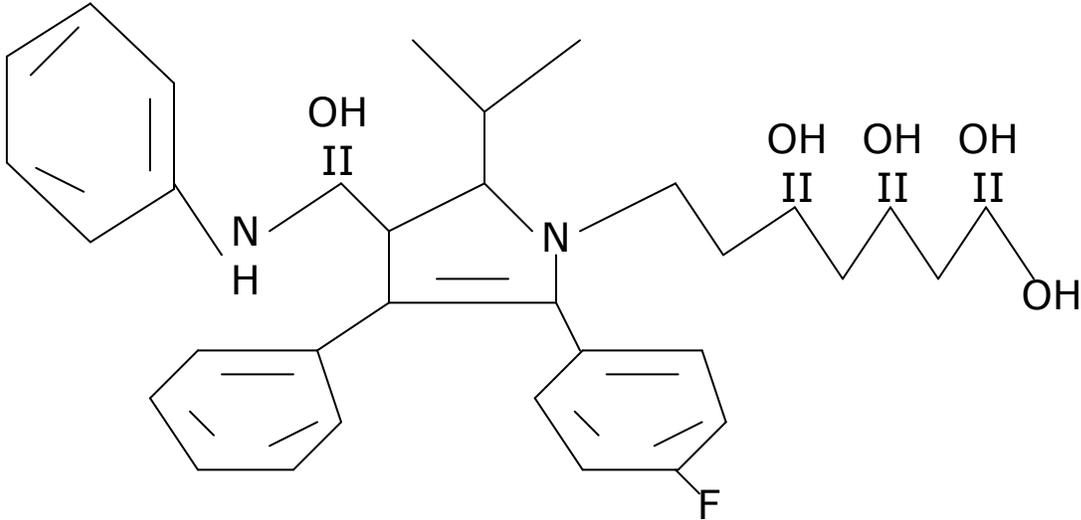
Familial combined hyperlipidemia is another disorder exacerbates the condition of lipoprotein and its mechanism is by increase in hepatic secretion of apolipoprotein B-containing VLDL and conversion to LDL. Accumulation of VLDL, LDL, or both depending on efficiency of their removal. It can lead to stroke, PVD and CAD.

Remnant removal disease (familial dysbetalipoproteinemia), increased secretion of VLDL, impaired removal of remnant lipoproteins resulting from homozygosity (E_2/E_2) or heterozygosity (E_2/E_3 or E_2/E_4) for apolipoprotein E E_2 and can lead to PVD, CAD and stroke.

Polygenic hypercholesterolemia is a lipoprotein disorder which causes the diminishing of LDL receptor activity and defective to apolipoprotein B that is poorly recognized by LDL receptor and leads to CAD, occasionally PVD, and stroke

2.4 ANTILIPIDEMIC DRUG ATORVASTATIN (LIPITOR)

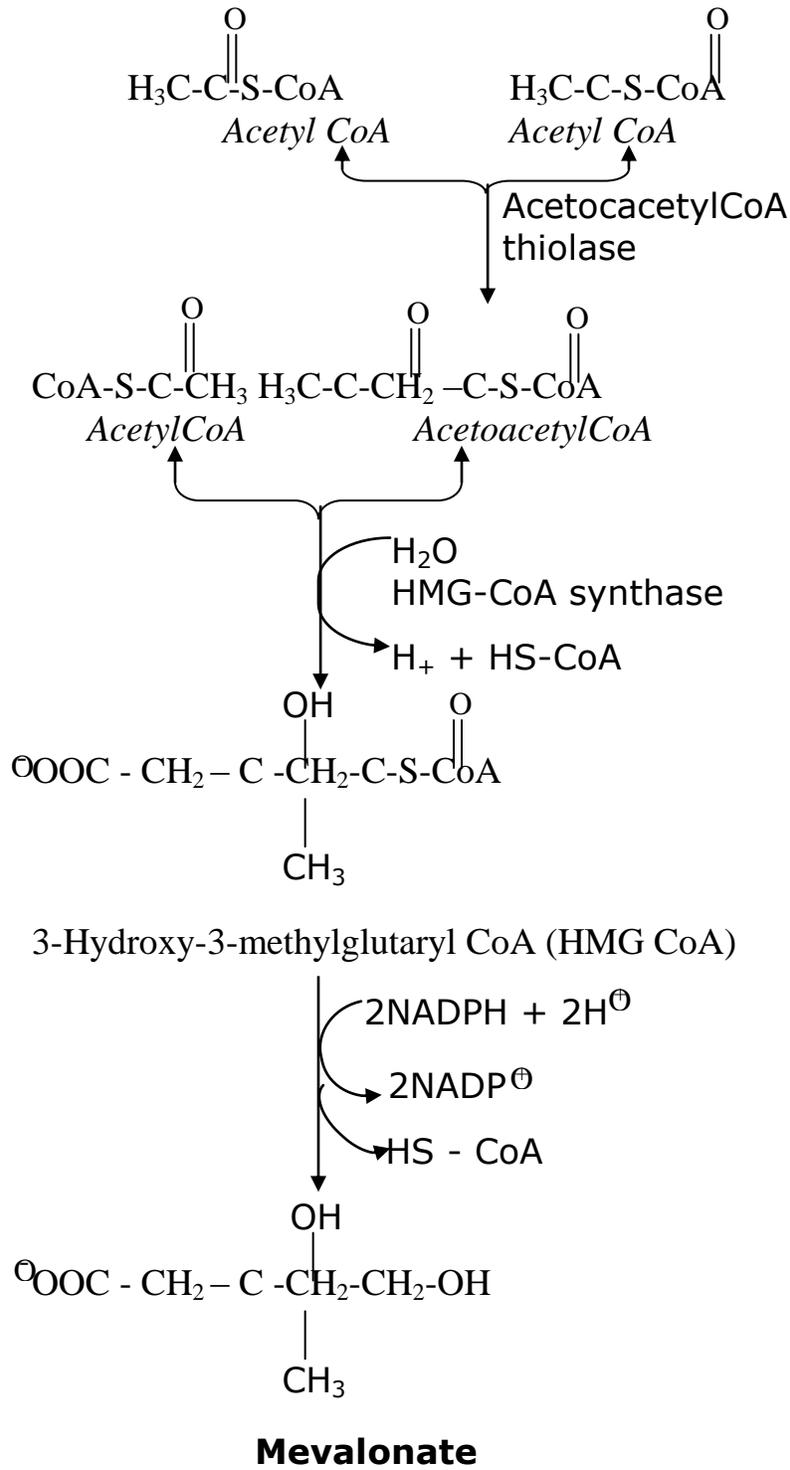
Fig. 1:



(3R, 5R) – 7-[2-(4-Fluorophenyl) -3-phenyl-4- (phenyl carbamoyl) -5-propan -2-ylpyrrol-1-yl]-3, 5-dihydroxy heptanoic acid.

Atorvastatin marketed by pfizer as a calcium salt under the trade name lipitor, is a member of the drug class known as statin, used for lowering blood cholesterol. It also stabilizes plaque and prevents strokes through anti-inflammatory and other mechanism. Like all statins, atorvastatin works by inhibiting HMG-CoA reductase, an enzyme found in liver tissue that plays a key role in production of cholesterol in the b

Mechanism of Action:Fig. 2:



As with other statins, atorvastatin is a competitive inhibitor of HMG-CoA reductase. It is a completely synthetic compound. HMG-CoA reductase catalyzes the reduction of 3-hydroxy-3-methyl glutaryl – Co-enzyme A (HMG-CoA) to mevalonate, which is the rate-limiting step in hepatic cholesterol biosynthesis. Inhibition of the enzyme decreases de novo cholesterol synthesis, increasing expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes. This increases LDL uptake by the hepatocytes, decreasing the amount of LDL-cholesterol in the blood.

It also reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol.

Medical Uses:

Atorvastatin is used for the treatment of dyslipidemia and the prevention of cardiovascular disease. It is recommended to be used only after other measures such as diet, exercise and weight reduction have not improved cholesterol levels.

Contraindications:

Active liver disease like cholestasis, hepatic encephalopathy, hepatitis and jaundice unexplained elevations in AST or ALT levels and also pregnancy or breastfeeding mother.

Precaution must be taken when treating with atorvastatin, because rarely may it lead to rhabdomyolysis, and it may be very serious leading to acute renal failure due to myoglobinuria.

Dosing:

Atorvastatin is prescribed once daily. The usually starting dose is 10-20mg per day, and the maximum dose is 80mg per day. Individuals who need more than a 45% reduction in LDL cholesterol may be started at 40mg daily. Atorvastatin may be taken with or without food and at any time of day.

2.4.1 OTHER ANTILIPIDEMIC DRUGS:

Statins are useful in treating most of the major types of hyperlipidemias but there are some other drugs which include fibrates, bile-acid-binding resins, ezetimibe, Niacin (Nicotin Acid) etc.

Ezetimibe:

Ezetimibe inhibits cholesterol absorption in the small intestine. This reduces absorption of dietary cholesterol, but also promotes cholesterol excretion, since biliary cholesterol accounts for some of the cholesterol that passes through the small intestine. Ezetimibe effectively lowers LDL cholesterol.

However, clinical trials have called in to question whether further lowering cholesterol with this drug is truly beneficial in reducing atherosclerosis and heart disease.

Fibrates:

Fibrates are the most effective triglyceride-lowering drugs. Fibrates enhance the oxidation of fatty acid (FA) in liver and muscle and reduce the rate of lipogenesis in the liver, thereby reducing hepatic secretion of very-low-density lipoprotein (VLDL), triglycerides. The increased uptake of triglyceride derived fatty acids in muscle cells results from an increase in lipoprotein lipase (LPL) activity in adjacent capillaries and a decrease in the apolipoprotein c-III(APO C-III) concentration mediated transcriptionally by peroxisome proliferators-activated receptor alpha ($PPAR\alpha$). The decrease in apo C-III reduces the inhibition of LPL activity.

The enhanced catabolism of VLDL generates surface remnants, which are transferred to high-density lipoprotein (HDL). HDL concentrations are further augmented by an increase in $PPAR\alpha$ -mediated transcription of apoA-I and apo A-II. The rate of HDL-mediated reverse cholesterol transport may increase. Fibrates activate $PPAR\alpha$, which binds to a $PPAR\alpha$ response element in conjunction with the retinoid X receptor. Other effects of fibrates include an increase in the size of LDL particles, increased removal of LDL and a reduction in the levels of plasminogen activator inhibitor type I.

Bile-Acid-Binding Resins:

Bile-acid-binding resins are cholestyramine and colestipol. Resins bind bile acids (not cholesterol) in the intestine, thereby interrupting the enterohepatic circulation of bile acids and increasing the enterohepatic circulation of bile acids in liver. Hepatic synthesis of cholesterol is also increased, which in turn increase the secretion of VLDL in to the circulation, raises serum triglyceride concentration, and limits the effect of the drug on LDL cholesterol concentrations. The increase in serum triglyceride concentrations can represent a major complication in patient who are prone to hypertriglyceridemia.

A bile-acid-binding resin is to reduce serum LDL cholesterol concentrations in patients who are already receiving a statin. The statin-induced inhibition of cholesterol synthesis increases the efficacy of the bile-acid-binding resin. In addition, serum HDL cholesterol concentration increases by about 0.5mg per deciliter (0.04mmol per liter) when a bile-acid-binding resin is added to the treatment regimen of patients who are already receiving a statin.

Niacin (Nicotinic Acid):

Niacin is an essential nutrient of the vitamin B complex and is the most effective drug for raising HDL levels. Nicotinic acid inhibits the

mobilization of free fatty acid (FFA) from peripheral adipose tissue to the liver. As a consequence of this decrease or an additional hepatic effect, the synthesis and secretion of very-low-density lipoprotein (VLDL) are reduced, and the conversion of VLDL to low-density lipoprotein (LDL) is decreased.

Nicotinic acids can also increase serum high-density lipoprotein (HDL) cholesterol concentrations by up to 30 percent; the mechanism responsible for this effect is unknown but it appears to inhibit an enzyme in the liver that is involved in triacylglycerol synthesis, causing a decrease in VLDL production.

2.4.2 OTHER THERAPIES:

Dietary supplementation with soluble fiber, such as psyllium husk, oat bran, gum and pectin, and fruit and vegetable fibers, lowers serum LDL cholesterol concentration by 5 to 10 percent. Sitostanol, a plant sterol incorporated into margarine inhibits gastrointestinal absorption of cholesterol. The n-3 fatty acids can lower serum triglyceride concentration by up to 30 percent at a daily dose of 3g and by about 50 percent at a daily dose of 9g.

In postmenopausal women, oral estrogen therapy can lower serum LDL cholesterol concentration by approximately 10 percent and raise serum

HDL cholesterol concentration by about 15 percent. Also an anabolic steroid such as oxandrolone or stanozolol is used to reduce the hepatic secretion of triglycerides.

2.4.3 HEALTH SIDE EFFECTS OF ATORVASTATIN:

Atrovastatin is generally well-tolerated. Minor side effects include constipation, diarrhea, fatigue, gas, heart burn and headache. Atrovastatin may cause liver and muscle damage. Serious liver damage caused by statins is rare. Liver tests should be performed at the beginning of treatment then as needed thereafter.

Inflammation of the muscle caused by statins can lead to serious breakdown of muscle cells called rhabdomyolysis. Rhabdomyolysis causes the release of muscle protein (myoglobin) in to the blood, and myoglobin can cause kidney failure and even death.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

The materials used during identification and extraction, phytochemical analysis and experimental animal model are:

Materials	Manufacturers
Beakers	Pyrex
Test tubes	Pyrex
Pipettes	Pyrex
Measuring cylinder	Pyrex
Electric grinder	Moulinex, 2000 France
Soxhlet apparatus	Pyrex
Spectrophotometer	Pee, medicals USA
Centrifuge	Haracus Christ
Refrigerator	Thermacool
Weighing balance	Camry, China
Filter papers	Whatman
Water bath	Griffens
Lab mortar	Gallen kamp
Dissecting kit	Vernex, medicals

Syringe	Changzhou,medicals china
EDTA tubes	
Hand glove	Jinxiang
Nose masks	Jinxiang
Cage	Local made

3.11 CHEMICALS AND REAGENTS

The chemicals used during extraction, phytochemical analysis and experimental animal model are:

Ethanol

Hydrogen chloride

Petroleum ether

Ethyl acetate

Ammonia slake

Phosphomolybdic acid

Formaldehyde

Methanol

Potassium ferricyanide

Alkaline picarate solution

Picric acid

Chloroform

Alkaline copper reagent

Colour reagent

Distilled water

Atorvastatin drug (Lipitor)

3.2 PLANT MATERIAL-COLLECTION AND IDENTIFICATION:

Healthy fresh leaves of *Desmodium velutinum* were harvested at Independence Layout, Enugu in the month of February 2013 from Prof. J.C. Okafor's garden, a taxonomist with the Department of Biotechnology, Enugu State University of Science and Technology, (ESUT) Enugu. The leaves were also authenticated by him – Prof. J. C. Okafor.

3.3 EXTRACTION:

The leaves were dried at a room temperature for eighteen (18) days. The dried leaves were later ground in to fine powder with the aid of a clean dry electric grinder (moulinex, optiblend 2000, made in France). A 130g portion of the ground leaves was soaked in 130ml of distilled water by hot-continuous percolation method in a soxhlet, (this is a continuous procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts and the finely ground crude is placed in a porous bag or thimble made of strong filter paper, which is place in chamber E of

the soxhlet apparatus). The water solvent in the extract was then distilled off in a distillatory and evaporated to dryness at 40^{0C}.

The solid extract weighing 18.3g, was placed in a sterile container labeled and stored at 40^{0C} 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 used for 12g was used for phytochemical analysis.

3.4 PHYTOCHEMICAL ANALYSIS:

Preliminary phytochemical test as described by Harbone (1973) and Trease and Evans (1996) were carried out on the solid extract of *Desmodium velutinum*. In general, tests for presence or absence of phytochemical compounds using the above methods involved the addition of an appropriate chemical agent to the solid extracts of the plant in a test tube. Summary of the methods are as below:

Analysis for Steroid:

About 20mls of ethanol was added to 1g of the extract to macerate and was filtered. 2mls of the filtrate was pipette and 2mls of colour reagent was

added and allow standing for 30 minutes, measuring the absorbance at 550nm.

Analysis for Saponin:

Weigh 1g of the extract it was macerate with 10mls of petroleum ether. Decant into a beaker and add another 10mls of petroleum ether. Decant it into the beaker combine the filtrate, allow to evaporate to dryness and 6mls of ethanol was added. Pipette 2mls into a test tube and add 2mls of colour reagent. Allow it to stand for 30 minutes and measure the absorbance at 550nm.

Analysis for Flavonoids:

About 20mls of ethyl acetate was added to 1g of the extract to macerate filter and pipette 5mls of the filtrate, add 5mls of dilute ammonia slake. Collect the upper layer and measure the absorbance at 49nm.

Analysis for Reducing Sugar:

About 20mls of distilled water was added to 1g of the extract to macerate and was filtered. Pipette 1ml of the filtrate, add 1ml of alkaline copper reagent and boil for 5minutes, allow to cool. Add 1ml of phosphomolybdic acid reagent and 7mls of distilled water, measure the absorbance of 420nm.

Analysis for Alkaloid:

About 20mls of 20% H₂SO₄ in ethanol (1:1) was added to 1g of the extract to macerate and was filtered. Pipette 1ml of the filtrate and add 5mls of 60% H₂SO₄ and 5mls of 0.5% formaldehyde in 60% H₂SO₄. Mix and allow to stand for 3 hours and measures the absorbance at 565nM.

Analysis for Terpenoid:

About 50mls of ethanol was added to 1g of the extract to macerate and was filtered. Pipette 2.5mls of the filtrate; add 2.5mls of 5% aqueous phosphomolybdic acid solution and 2.5mls of conc H₂SO₄ gradually. Mix and allow to stand for 30minutes, make up to 12.5mls with ethanol and measure the absorbance at 700nM.

Analysis for Glycoside:

About 2.5mls of 15% lead acetate was added to 1g of the extract to macerate and was filtered. 2.5mls of chloroform was added and shake vigorously, collect the lower layer and evaporate to dryness. Add 3mls of glacial acetic acid and 0.1ml of 5% ferric chloride and 0.25ml conc H₂SO₄ and shake. Put in the dark for 2 hours and measure the absorbance at 530nM.

Analysis for Tannin:

About 50mls of methanol was added to 1g of the extract to macerate and was filter. Pipette 5mls of the filtrate and add 0.3mls of 0.1M ferric chloride

in 0.1M HCL and 0.3mls of 0.0005M potassium ferricyanide. Measure the absorbance at 720nm.

Analysis for Cyanide:

About 50mls of distilled water was added to 1g of the extract to macerate and stand for 24hrs. Filter and pipette 1ml of the filtrate add 4mls of alkaline picrate solution and boil for 5 minutes. Allow to cool and measure the absorbance at 490nm.

Analysis for Soluble Carbohydrate:

About 50mls of distilled water was added to 1g of the extract to macerate and was filter. Pipette 1ml of the filtrate and add 2mls of saturated picric acid and measure the absorbance at 530nm.

3.5 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 500mls of distilled water forming a semi-solid mixture. A known antilipidemic drug; atorvastatin (brand name-lipitor, 10mg) was prepared by

dissolving 5mg (half of one tablet) in 2ml of distilled water. Also *Desmodium velutinum* leaves water extract weighing 6.3g was dissolved in 17mls of distilled water forming a liquid drug extract.

Group I rats were fed orally with only growers mash and water for also seven (7) days.

Group II rats were fed orally with 6mls of the lipoprotein food mixture which contains 3.6g of the cow's brain twice a day (morning and evening) for seven (7) days.

Rats in group III were also fed orally with 6mls of the lipoprotein food mixture for seven (7) days (morning and evening) and later were administered orally with the 2mls dissolved atorvastatin drug for the following three (3) days (once each day) during which growers mash and water was their food.

Rats in group IV were also fed orally with 6mls of the lipoprotein food mixture for seven (7) days (morning and evening) and later were administered orally with 0.5ml of the liquid drug extract. (*Desmodium velutinum* leave water extract mixture) for the following three (3) days (once a day) during which growers mash and water was their food.

NB: Both the lipoprotein food mixture and the extract doses were orally administered by the use of a syringe (needle part removed).

Nutritional value of the grower's mash (Guinea feed Nigeria)

Crude protein.....	Min 20.00%
Crude fat.....	Min 2.00%
Crude fiber.....	Max 4.5%
Calcium.....	Actual 1.00%
Phosphorous.....	Actual 0.75%
Sodium.....	Actual 0.18%
Vitamin A.....	Min 12000 IU/kg
Vitamin D3.....	Min 2000 IU/kg
Vitamin E.....	Min 20 IU/kg

Source: Guinea Feed Nigeria Label .Min- Minimum, Max- Maximum

Nutritional content of raw cow's brain

Water.....	76g in 100g of cow's brain
Protein.....	10.9g in 100g of cow's brain
Fat.....	10.3g (2.3g is saturated fat) in 100g of cows brain .most of the is located in the myelin (which itself is 70-80 fat)
Carbohydrate.....	1g in 100g of cow's brain

There is no fiber or sugar content in cow's brain.

Source: United State Dietary Association, 2010

3.6 COLLECTION OF BLOOD SAMPLES:

The collection of blood samples from the rats in each group was simply done by dissecting of the rats, following by cardiac puncture after a mild anesthesia 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.

Blood samples were collected from group I rats and group II rats on the following day of after the 7th day of orally feeding the rats with lipoprotein food mixture and normal feed (growers mash and water) respectively.

Blood samples were collected from group III and IV rats on the following day of after the 3rd day of orally administering a known drug (atorvastatin, Liptor) and the liquid drug extract (*Desmodium velutinum* leave water extract mixture) respectively.

3.7 LIPID PROFILE ANALYSIS:

In the lipid profile analysis, test is 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.

Test for Total Cholesterol (TC):

(a) Material/Reagents involved

- Serum
- Ferric chloride reagent (2.5g of ferric chloride mixed with 100mls of 85% phosphoric acid).
- Standard cholesterol (250mg of cholesterol mixed with 100mls 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 labeled –Test, Standard and Blank respectively, the following procedures was conducted.

Table 2:

Sample	Test	Standard	Blank
Ferric Chloride Reagent	5.0mls	5.0mls	5.0mls
Diluted Serum	0.5mls	-	-
Diluted Standard Cholesterol	-	0.5mls	-
Distilled Water	-	-	0.5mls

- 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.

Meanwhile, the colour should be stable for 15minutes.

- Finally, calculate using the formular:

$$\frac{\text{Absorbance Test}}{\text{Absorbance Standard}} * 250(\text{constant value})$$

The unit is mg/dl

Then to convert the result from mg/dl to mmol/l simply multiply the mg/dl result by 0.0259 (constant value).

Test for HDL-Cholesterol (HDL-C):

Material/Reagents involved

- Serum
- Cholesterol standard (100mg of pure cholesterol mixed with 100mls 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).
- Magnesium chloride (40.6g of magnesium chloride mixed with 100ml of water).

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

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 30minutes.
- 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.
- Finally, calculate using the formula:

$$\text{HDL-C} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} * 155(\text{constant value})$$

Test for LDL –Cholesterol (LDL-C):

The LDL-Cholesterol is calculated using the standard formula:

$$\text{Total Cholesterol} - \text{HDL-Cholesterol} + 0.46(\text{constant value})$$

Test for Triglycerides (TG):

(a) **Material/Reagents involved**

- Serum

- Heptane
- Isopropanol
- Sodium methylate (50mg of sodium methylate diluted in 100mls of isopropanol)
- Sulphuric acid (0.08N)- prepared by mixing 2.25mls conc H₂SO₄ of about 36.0N with 500ml of distilled water. The dilution should be up to 1 litre.
- Periodate reagents (1.23g) of NaO₄ was mixed with 100mls of 0.88N, about 5% V/V/ action acid. Then stored in a brown bottle)
- Acetylacetone reagent (0.75mls of acetyl acetone was dissolved with 2.5mls 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^{0C}.
- 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.

Table 3:

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

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

- Shake the tubes for 30 seconds to mix very well
- The tubes should stand for 10minutes at room temperature for proper separation of two layers
- Prepare another set of three test tubes labeled-Test, Standard, and Blank respectively and the following procedures was conducted:

Table 4:

Sample	Test	Standard	Blank
Top Solvent Layer from respective tube	0.2ml	0.2ml	0.2ml
Sodium Methylate	3.0mls	3.0mls	3.0mls

- Shake the tubes using vortex mixer to mix very well.
- Incubation at 60⁰C for 10minutes 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 10minutes of incubation at 60⁰C.
- Centrifuge the tubes and transfer the upper phase liquid to another fresh tube.

- Using spectrophotometer, read the absorbance of test and standard at 420nm against blank.
- Finally calculation is done by:

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

Then to convert to mmol/dl is mg/dl *0.0113(standard value)

CHAPTER FOUR

STATICALLY ANALYSIS

Statically analysis was carried out using statically package for social sciences

PHYTOCHEMICAL RESULTS:

The modified Harbourne's (1973) phytochemical analysis revealed the presence of soluble carbohydrate, cyanide, sugar, saponin, tannin, flavonoids, alkaloids, steroids, terpenoids, in the extract in different concentration (table 1 and 2).

Table 1:

Quantitative analysis of phytochemical composition of water extract of leaves of DV and other samples (mg/100g)

SAMPLE	SOLUBLE CHO	CYANIDE	REDUCING SUGAR	SAPONIN	TANNIN	FLAVON OIDE	ALKALON IOD	STERIOD	TERPENOI DE
Water Leaf Extract of DV	1.43 ± ^a 0.003	0.63 ± ^a 0.003	321.743 ± ^c 0.003	1.05 ± ^c 0.003	2.87 ± ^c 0.004	3.82 ± ^a 0.003	3.78 ± ^c 0.089	0.63 ± ^c 0.004	0.28 ± ^c 0.005

Data are means of triplicate determinations ± standard deviation (SD)

Data in the same column bearing different superscript differed significantly (P ≤ 0.005).

Table 2:

Qualitative analysis of phytochemical composition of water extract of leaves
of DV and other samples

Tannin	+++
Alkaloid	+++
Carbohydrate	+
Saponin	+
Steroid	+
Hydrogen Cyanide	+
Flavonoid	++
Reducing Sugar	++
Terpenoid	+

Data:

- + = Present,
++ = Strongly present,
+++ = Fully Present

The effect of fresh cow's brain, atorvastatin and water extract of
D.velutinum on albino wistar rats is shown in table 3.

Table 3:

Lipid profile of rats fed with various samples (mg/dl)

Rats (Samples)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglyceride (mg/dl)
Group 1 (Normal Feed)	140.00 ± 1.41 [*]	30.00 ± 1.41 [*]	3.60 ± 0.14 [*]	95.00 ± 1.41 [*]
Group 2 (Cow's Brain)	145.00 ± 1.41 [*]	40.00 ± 1.41 [*]	3.90 ± 0.14 [*]	105.00 ± 1.41 [*]
Group 3 (Cow's Brain)	110.00 ± 0.00 [*]	13.00 ± 1.41 [*]	1.20 ± 0.14 [*]	39.00 ± 1.41 [*]

+ Atorvastatin)				
Group 4 (Cow's brain + Water extract of DV leaf)	135.00 ± 0.71*	25.00 ± 0.00*	1.90 ± 0.00*	50.00 ± 0.00*

Data are means of duplicate determinations ± standard Deviation (SD) – Data in the same column bearing asterix (*) are significantly different (P<0.05)

In group 1, animals, the normal feed, the LDL (3.60 ± 0.14 mg/dl) and HDL (30.00 ± 1.41 mg/dl), in group 2, the LDL significantly increased to 3.90 ± 0.14 mg/dl and HDL increase to 30.00 ± 1.41 mg/dl because their were fed with high lipoprotein food.

In the atorvastatin treated (2ml) group the LDL level was found to be 1.20 ± 0.14 mg/dl decrease and HDL to 13.00 ± 1.41 mg/dl decrease compared with the group treated with the water extract of *D.velutinum* (0.5ml) exhibited a progressively decrease in LDL to 1.90 ± 0.00mg/dl by inhibiting the HMG-CoA and significantly increased the HDL to 25.00 ± 0.00mg/dl.

CHAPTER FIVE

DISCUSSION AND CONCLUSION:

This study evaluated the antilipidemic activity of the water extract of *Desmodium velutinum* in albino wistar rat. Hyperlipidemia comprises a state of increased concentrations of TG, TC and LDL-C and is an important risk factor for the development and progression of atherosclerosis and coronary heart diseases (Viran *et al.*, 2012).

In table 1, the data shows that, there is a significantly high content of alkaloid, reducing sugar, soluble carbohydrate, flavonoid, and tannin in water extract of D.V. leaf. These alkaloids often have pharmacological effect and are used as medication, as recreational drug, exert anti-asthma, anti-cancer, anti-arrhythmia (Manske, 2006) and reduce unwanted side effects. Tannin compounds are biomolecule, as in an astringent, bitter plant polyphenolic compounds that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids (Kadam *et al.*, 2005). Tannins and alkaloid may also be effective in reducing the risk of cardiovascular disease. The content of cyanide present in water extract of D.V leaf is very small. Even though cyanide is said to be toxic to the body, its content in the extract is minimal (0.63 ± 0.003) thus opposes no threat to the body.

In table II shows the presence of flavonoids, saponin, soluble carbohydrate, cyanide, terpenoid, steroid, reducing sugar, alkaloid and tannin, in extract of D.V. leaf .But tannin and alkaloid showed fully present, flavonoid and reducing sugar showed strongly present significantly. Flavonoids are most commonly known for their antioxidant, anti-inflammatory, anti-microbial, anti-allergic, anti-cancer and anti-diarrheal activities. (Cushine and Lamb, 2005). Flavonoids have medicinal properties especially their putative role in inhibiting cancer and cardiovascular disease (Frei, 2006).

Saponin involves the complexation with cholesterol to form pores in cell membrane bilayer, e.g., in red cell (erythrocyte) membranes, where complication leads to red cell lysis on intravenous injection (Yah *et al.*, 2010) and also show antioxidant, anticancer activity. Saponin have shown to reduce cardiovascular and atherosclerosis. Polysaccharide and protein (e.g., glycoprotein complexes present in plant extracts (Oh *et al.*, 2006) could also be involved in the hypolipidemic and antiatherosclerotic effects of *D.velutinum*. Therefore, the association between these complexes and compounds and other constituents may play an important role in the biological activity of the leaf. Soluble carbohydrate is said to help control

body weight lower blood glucose and cholesterol (mayor, 2011) by inhibiting the absorption of fats and cholesterol in intestine.

In table II, the group 1 fed normal feed the LDL.C is 3.60 ± 0.14 and HDL.C. is 30.00 ± 1.41 . In group II fed with cow's brain effectively increase plasma lipid significantly to LDL-C 3.90 ± 0.14 and HDL-C 40.00 ± 1.41 , thereby causing an increase in the activity of HMG-CoA reductase and acting on cholesterol synthesis.

In group II treated with atorvastatin reduces the plasma lipid significantly to LDL-C 1.20 ± 0.14 and HDL-C 13.00 ± 1.41 which can possibly leads to hypocholesterolemia compared to group IV treated with water extract of D.V. leaf reduce plasma lipid to LDL-C 1.90 ± 0.00 and an increase in HDL-C 25.00 ± 0.00 which is the good cholesterol. The data demonstrate that water extract of D.V. leaf can possibly normalize the plasma lipid. The study suggests that the water extract of the leaf is effective in reducing lipid plasma, thereby reducing the risk of cardiovascular and atherosclerosis disease. The findings lend support to the folkloric use of D.velutinum in the Eastern Nigeria as an antilipidemic agent.

5.2 CONCLUSION:

The water extract of *Desmodium velutinum* leaves showed significant antilipidemic effect in experimental rats.

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