BIOACTIVITY-GUIDED FRACTIONATION OF AQUEOUS LEAF EXTRACT OF*TEPHROSIA VOGELII* AND ITS EFFECT ON HYPERLIPIDEMIA IN RATS

BY

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DECLARATION

I hereby declare that the work in this thesis titled "Bioactivity-guided Fractionation of Aqueous Leaf Extract of*Tephrosia vogelii* and its Effect on Hyperlipidemic in Rats " has been carried out by me in the Department of Biochemistry. The information derived from the literature has been dully acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree at this or any Institution.

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Name of Student Signature Date

CERTIFICATION

This thesis entitled "Bioactivity-guided Fractionation of Aqueous Leaf Extract of*Tephrosia vogelii* and its Effect on Hyperlipidemic in Rats" by Inalegwu BAWA meets the regulations governing the award of the degree of Doctor of Philosophy of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicated this work to God Almighty for giving me life and for sustenance and my late mother, Omeyi Inalegwu.

ACKNOWLEDGEMENT

I remain grateful to God whom am indebted to, with my entire life for keeping me alive throughout my study in this University and the also for the gift of knowledge and vision as well as whom I recognize as my divine source, from whom I tapped all the wisdom, vision and favour for the completion of this work. I thank you, Lord.

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ABSTRACT

There are claims by traditional medicine practitioners in Nigeria that the leaves of *Tephrosia vogelii*hook. f. are used for the treatment of cardiovascular diseases but it appears there is no scientific research and publication or documented work to verify these claims.Thisstudy aimed for bioactivity guided fractionation of aqueous leaves extract of*T. vogelii*and its effect on hyperlipidemic rats. Induction of hyperlipidemia was carried out with high fat diet and Poloxamer 407 (P-407). Partial purification and identification were carried out using Thin Layer Chromatography (TLC), Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography- Mass Spectra (GC-MS). The results of the phytochemical screening of leaves and stem revealed the presence of alkaloids, saponins, phlobatannins and flavonoids in powdered sample of leaves and stem of *T. vogelii*.Aqueousextract of the leaves has the highest yield (18.21±1.12%) while acetic acid extract of stem has the lowest yield (7.21±1.21%). The cholesterol and triacylglycerol level of rats induced with P-407 was significantly ($p<0.05$) higher than high fat diet-induced rats. Preliminary antihyperlipidemic study showed aqueous extract at 50mg/kg body weight significantly ($p<0.05$) lower cholesterol, low density lipoprotein (LDL) and triacylglyceride levels. The $LD_{50}$of aqueous leaves extractwas found to be 2154mg/kg body weight.Ten fractions (F1-F10) wereobtained from methanol in water leaves extract of *T. vogelii* significantly ($p<0.05$) reduced total cholesterol (TC) when compared with normal and hyperlipidemic control.Fraction six (F6) (100mg/kg body weight) significantly ($p<0.05$) reduced TC, TAG and LDL-c, AST, ALT, MDA, creatinine, total bilirubin and urea and significantly ($p>0.05$) increase SOD, CAT and GSH when compared with extract treated group and hyperlipidemic group. Lipid profile

of F6 was significantly ($p<0.05$) lower than normal rats.There was no significant ($p>0.05$) change in the level of TC, HDL at a higher dose. Serum TAG, LDL-c, ratio of LDL-c/HDL and Log TAG/HDL-c were significantly ($p<0.05$) reduced while ratio of HDL-c/TC was significantly ($p<0.05$) higher. The TLC separation in solvent system of hexane/ethylacetate in ratio 7:3 gave two fractions (subfraction 1 and 2) with Rf values of 0.22 and 0.60 respectively.Antihyperlipidemic effect of subfraction1 (Rf value 0.22) significantly ($p<0.05$) reduced triacylglycerol (TAG) when compared to animal treated with other *T. vogelii* fractions and show no significant change when compared with standard drug and animal in control group. There was no significant ($p> 0.05$) change in the level of total cholesterol (TC), low density lipoprotein (LDL), and high density lipoprotein (HDL) infraction 6, subfraction 1 and 2 when compared with hyperlipidemic control group. The FTIR of subfraction1 showed the presence of phenols, alcohols, alkanes, alkyl halides, carboxylic acids, aromatics, nitro compounds and amines. The prevailing functional groups were alcohol and phenol.The GC-MS analysis of subfraction1shows the presence of fourteen compounds. The prevailing compounds were gamma.-Tocopherol and other compounds that have antihyperlipidemic effect including 4H-pyran-4-one, 3, 5-dihydroxy, 2-Methyl-Z, Z-3, 13-octadecadienol and 9-Octadecenoic acid, (E). The study shows that the aqueous extract ofleaves possess antihyperlipidemic effects and may explains why it has been found to be useful in the management of cardiovascular diseases by traditional medicine practitioners.

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LIST OF ABBREVIATIONS

%: Percentage

µl: Microlitre

mg: Milligram

^{0}C: Degree Celsius

hr :Hour

Da: Dalton

kDa :killo-Dalton

LD_{50}: Lethal Dose at 50 percent

TLC:Thin Layer Chromatography

GC/MS:Gas Chromatography Mass spectroscopy

ALP: Alkaline Phosphatase

ALT: Alanine Aminotransaminase

ANOVA: Analysis of Variance

AST: Aspartate Aminotransaminase

CVD: Cardiovascular Disease

DPPH: 2, 2-diphenyl-1-picrylhydrazyl

HDL-c: High-Density Lipoprotein cholesterol

HMG-CoA: 3-hydroxy-3- methylglutaryl-CoA reductase

LCAT: Lecithin Cholesterol Acyltransferase

LDL-c:Low-Density Lipoprotein cholesterol

NCEP: National Cholesterol Education Programme

P407:Poloxamer 407

SGOT: Serum Glutamic Oxaloacetic Transaminase

SGPT Serum Glutamic Pyruvic Transaminase

SOD Superoxide Dismutase

TAG Triacylglycerides

CHEPTER ONE

1.0 INTRODUCTION

Cardiovascular diseases (CVDs) such asmyocardial infarction (heart attacks), coronary heart disease, high blood pressure, peripheral vascular diseases and stroke are the leading causes of death for both men and women among all racial and ethnic groups (Smith, 2004). They lead to nearly 50% of all deaths in the developed world (Thomas and Rich, 2007; Mendis *et al*., 2011). If this continues, the future will even be more challenging considering that globalization and wide spread of western diet to the developing world resulting to high rates of obesity and hyperlipidemia in developing countries (Lim *et al.*, 2012). Thus, hyperlipidemia and its associated CVDs are considered as one of the highest worldwide economic, social and medical challenges (Olshanky *et al*., 2005). The elevation levels of serum total cholesterol and more importantly low density lipoprotein (LDL) cholesterol have been implicated as primary risk factor for cardiovascular disease (Edijala *et al*., 2005).Young *et al*., (2012) shows that rats can be hyperlipidemically induced by poloxamer 407 (HL rats).

Control of cholesterol levels through therapeutic drugs have significantly reduced the risk of developing atherosclerosis and associated cardiovascular diseases (Khush and Waters, 2006; Stacy and Egger, 2006; Ray *et al*., 2006). Statins are notablya class of hypolipidemic drugs inhibiting cholesterol synthesis. They have been used as the most widely prescribed for treating hypercholesterolemia and reducing cardiovascular diseases (Ray *et al*., 2006; Khush and Waters, 2006). However, adverse effects associated with hypolipidemic drugs, such as liver damages myopathy and potential drug-drug interaction have been

reported (Parra and Reddy, 2003; Neuvonen *et al*., 2006; Trifiro, 2006; Kiortsis *et al.,* 2007). As the result, development of additional therapies for controlling cholesterol levels with less or no adverse side effect such as plant derived products is justified, especially for those with better safety profile.

Medicinal plants have been used in many African countries and today almost every part of the world use herbal plants for treatment of different diseases (Adewunmi and Ojewole, 2004). Medicinal plant is any plant in which one or more of its organs, contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Schafer *et al*., 1997). Medicinal plants are the main sources of chemical substances with potential therapeutic effects. The use of medicinal plants for the treatment and management of many diseases is associated with folk medicine from different parts of the world. Medicinal plants are progressingly being used in most parts of the world as: anticonvulsant (Oyewole and Amabeoku, 2006), hypoglycemic and hypocholesterolemic (Brai*et al*., 2007), wound healing (Nayak*et al.*, 2008), analgesic and anti-inflammatory (Adeyemi *et al*., 2002) and reducing cancer risk (Lu *et al.*, 2005). Especially for those with cholesterol at the borderline levels, herbal remedies have increasingly become attractive alternatives to control hypercholesterolemia. Excellent safety profile, cost effectiveness and multiple beneficial effects on improving wellbeing, have collectively contributed to the emerging trend of increasingly usage of herbal supplements. Studieshave revealed that the reduction of LDL-cholesterol with different medicinal plants will reduce the incidence of the diseases associated with cardiovascular diseases and overall death rate (Schafer *et al*., 1997; Adebayo *et al.*, 2006).

Tephrosia vogelii Hook. f. belongs to the family Fabaceae, Genus *Tephrosia*and species *vogelii*. It is a native to Nigeria, Kenya, South Africa, Zambia, Tropical America, Southeast Asia and Malaysia as a cover crop. It is commonly called fish-poison bean in English and in Nigeria,*T. vogelii* is locally called *Oha* [Idoma] and *Kuhwa*[Tiv] as well as *Golon Zaki*[Hausa]. The plant is well known to the people of Ugboju and Okokolo Agatu. The plant is a soft woody branching herb or small tree with dense foliage, 0.5-4 m tall. Stem and branches tormentose with long and short white or rusty-brown hair. Leaves are arranged spirally, stipules 10-22x3-3.5 mm, ealy caducus; rachis 5-25 cm long, 1.5-5 mm long including petiolule; leaflets in 5-14 pairs. Flower 18-26 mm long, fragment when fresh, white, violet-purple or blue; pedicel up to 23 mm long, bracteoles sometimes present on calyx. The flowers are bisexual, born in compact clusters. It is a perennial shrub with mauve flowers and many pods. The commonly used parts of the plant include bark, fruit, roots, stem and leaves(Machocho *et al.,* 1995; Wanga *et al.*, 2006).

T. vogelii Hook (Fabaceae) has been used as a source of contact insecticides, fish and arrow poisons (Soko, 1999).This species helps in the restoration of soil fertility (Machocho *et al.*, 1995). It is maintained as a semi cultivated plant in dooryards in some primitive areas where it is used for poisoning fish because the plant's leaves, branches and seeds are used for stupefying fish, which remains fit for human consumption. The toxicity of *T. Vogelii* is lower than that of other plant species used in the industry. *T. vogelii* is traditionally used for its ichthyotoxic, insecticidal and food parasiticidal properties (Ibrahim *et al.*, 2000). Several isoflavonoids have been isolated from Tephrosia species the compounds isolated exhibited

different effects ranging from anti-feedant, antibacterial, insect-repellant and insecticidal (Machocho *et al.*, 1995). Small-scale farmers in Kenya are reportedly using the roots and leaves of *T. vogelii* to control diseases and pests on their crops (Wanga *et al*., 2006). Tephrosia is a potential source of rotenone, an important nonresidual insecticide, and tephrosin which is the main active compound useful in killing fish. The seeds are saved and planted, and the plants are tended but may also occur in the wild state. Although undocumented, the plant *T. vogelii* is used in South West Nigeria in the treatment of hypertension and other cardiovascular related diseases (Nworgu *et al.*, 2008).

1.1 Statement of the Research Problem

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases (Grundy, 1986; Senecha *et al*., 2012). Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death (Davey, 1993; Senecha *et al*., 2012). Hyperlipidemia is characterized by elevated serum total cholesterol and low density and very low density lipoprotein cholesterol and decrease high density lipoprotein are the risk factor for coronary heart diseases (Kumar *et al*., 2010; Rohilla *et al*., 2012). At same time cardiovascular death and disease have increased at an astonishing fast rate in low and middle income countries (Mendis *etal*., 2011). The World Health Organization estimates that this disease is responsible for the deaths of approximately 30,000 people each day (Middlemiss and Watson, 1994; American Heart Association, 2008).

Premature death caused by heart and blood vessels disease (CVD) in people of working age (35-64 years) are expected to increase by 41 percent between 2000 to 2030 and the

negative economic impact will be enormous (Leeder *etal*., 2004).Sequel to the wide spread of western diet high in saturated fats and cholesterol as well as other factors such as age, gender, lifestyle, stress, hereditary and hypertension to the developing world, there has been tremendous increase in the rates of obesity, overweight and hypercholesterolemia in the developing world which are mainly responsible for onset of coronary heart diseases (CHDs)(Choudhary *et al*., 2005; Zhao *etal*., 2005). Although there is a paucity of data on the prevalence of cardiovascular disease in Nigeria and other African countries, available data suggest that cardiovascular disease is emerging as major health problem in Africa, including Nigeria (Laakso, 1999).Control of cholesterol and triacylglycerol(TAG)levels through therapeutic drugs (mainly statins), have significantly decreased the risk of developing cardiovascular diseases(CVDs)(Stacy and Egger, 2006). However, currently available hypolipidemic drugs have been associated with number of side effects (Brown, 1996; Saravanakumar *et al.,* 2010). The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function (Speight, 9187; Saravanakumar *et al.,* 2010). In addition, the rural populations in various parts of the world do not have adequate access to high quality drugs for the treatment of cardiovascular diseases, so they depend heavily on plants and herbal products for the treatment of thediseases associated with cardiovascular diseases.

1.2 Justification of the Study

The presence of active components have been reported in more than 100 families of plants, and in a few marine sources such as star fish and sea cucumber as well as in beverages such as tea and red wine (Hostettmann and Marston, 1995). Leeder *etal*., (2004) and Duff,

(2008) suggest that diet rich in active compounds are associated with a decreased risk of cardiovascular diseases.

Researchers are showing interest in finding out an alternative medicine that is affordable, efficacious and with little or no side effects that will be available to low income earners for the treatment ofdiseases that are associated with cardiovascular diseases (Achinewu *etal*, 1995; Yokozawa *et al*., 2006). It is well established that man consumes a wide variety of local crops and vegetables, which are believed to contribute significantly to the improvement of human health in terms of disease prevention and therapy (Breazile, 1971; Burkill, 1985).

There are claims by traditional medicine practitioners that the leaves extract of *T. vogelii* are used for the management of diseases associated with CVDs but it appears there is lttle scientific research and publication or documented work to verify these claims. It becomes pertinent to determine the efficacy of this plant in the treatment of hyperlipidemia.Hence there is a need to investigate the effects of*T. vogelii* on hyperlipidemia in ratsfor the purpose of including it as herb for treatment of cardiovascular diseases.

1.3 Aim and Objectives of the Study

Aim:

The aim of this study was to carry out thebioactivity-guided fractionation of aqueous leaves extract of *T. vogelii* and its effect on hyperlipidemic rats in the management and treatment of hyperlipidemia.

The Specific Objectives are:

Specifically, the study seeks to;

i. Determine the phytochemical constituents (qualitative and quantitative) of the leaves and stem parts of the *T. vogelii* and evaluate the*in vivo* antihyperlipidemic activity of the extracts.

ii. To determine the effect of fractions from fractionation of the most potent aqueous leaves extract of *T. vogelii* on hyperlipidemic rats.

iii. Assess the effect of the aqueous extract (most potent) andfractions of*T. vogelii* on alkaline phosphatase (ALP), alanine transaminase (ALT), aspartatetransaminase (AST), total bilirubin,directbilirubin,total cholesterol (TC), triacylglycerol (TAG), high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C in hyperlipidemic rats.

iv. Evaluate the effect of the aqueous extract (most potent) and fractions of *T. vogelii* on the levels of endogenous antioxidants(superoxide dismutase, catalase) and TBARS in hyperlipidemic rats.

v. Identifypossible components from *T.vogelii* using TLC, FTIR and GC/MS.

CHAPTER TWO

Literature Review

2.1 Medicinal Plants

Natural products have been considered as a source of medicinal agents from the ancient time itself. An impressive number of modern drugs have been isolated from natural sources and the basis for the isolation is sometimes based on the traditional use of these plants in treatment of disease. This plant-based, traditional medicine system still acquires an important place in the health care system (Owolabi *etal*., 2007). Today, medicinal plants are increasingly being used in most parts of the world as hypolipidemic (Yadav *etal*., 2008), contraceptive, abortifacients, emmenagogues, or oxytocic, antihypertensive (Nworgu *etal.,* 2008), treatment for skin diseases, wound healers, antimicrobial, and hypoglycemic (Lee *etal*., 2009). The useful antihyperlipidemic phytochemicals include phenolics and polyphenols, quinones, flavonoids and flavones, tannins, coumarins, alkaloids, terpenoids and essential oils. The use of plant and animal parts for medicinal purposes has long been in existence and has been widely documented (Pomulo and Ierece, 2007). These ancient indigenous practices were discovered by a series of trial and error which then could not be substantiated by proven scientific theories. However, these practices have produced results of proven efficacies (Brai*et al*., 2007). In recent times herbal medicines have become indispensable and are forming an integral part of the primary health care system of many nations (Fajimi and Taiwo, 2005). Plants remain the

most common source of antihyperlipidemic agents. Their usage as traditional health remedies is most popular for 80% of world population in Asia, Latin America and Africa (Bibitha *etal*., 2002). In recent years pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants to produce cost effective remedies that are affordable to the population (Pandey and Singh, 2011). Similarly, there has been a constant increase in the search of alternative and efficient compounds for food preservation aimed at a partial or total replacement of antihyperlipidemicchemical additives (Buragohain *et al.*, 2012; Ranjan *et al.*, 2012). The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. Hence, a large number of medicinal plants are available.

2.2 Fish Poison Bean (*Tephrosia vogelii)*

2.2.1. Taxonomy

Kingdom: *Plantae*- Plant

Subkingdom: *Tracheobionta*-vascular plants

Subdivision: *Spermatophyta*-seed plants

Division: *Magnoliophyta*-flowering plants

Class: *Magnoliopsida*- dicotyledons

Subclass: Rosidae

Order: Fabales

Family: Fabaceae-Pea family

Genus: Tephrosia Pers-hoarypea

Species: *T. vogelii* Hook.F.-Vogel's tephrosia



Plate 2.1 Picture of *T. vogelii* hook f Plant (leaves and flowers).

2.2.2 Biological activity of *T. vogelii*

2.2.2.1Piscicidal activity

It is maintained as a semi cultivated plant in dooryards in some primitive areas where it is used for poisoning fish because the plants leaves, branches and seeds are used for stupefying fish, which remains fit for human consumption. *T. vogelii* has been used as a source of contact insecticides, fish and arrow poisons (Soko, 1999). Tephrosin is the main active compound used in killing fish. The toxicity of *T. vogelii*is lower than that of other plant species used in the industry. Exposure of *Tilapia zilli*to *T. vogelli* under laboratory conditions moderately affected respiratory distress, loss of balance, settling at the bottom motionless and erratic swimming (Akpa *et al.,* 2010). These conditions which may become aggravated in field application where lethal doses of the plant materials are employed in harvesting fish in many parts of Nigeria. The results obtained from the study implicated *T.vogelii* to be a poisonous plant hence the continuous use of the plant (*T. vogelii*) as fish bait by artisanal fishermen should be campaign against, as a result of its resultant negative effects on the quality of the water and the impairment of the body chemistry of the fishes due to bioaccumulation (Adewoye, 2010). It is evident that *T. vogelii* is toxic to *Tilapia zilli* and could possibly affect other aquatic animals. However, fishing methods which include the use of plants that contain toxic substances as active ingredients should be discouraged so to conserve the biodiversity of fish and other aquatic organisms in Nigeria water bodies(Akpa *et al.*,2010).

2.2.2.2Antibacterial activity

T. vogelii leaf extract (200mg/kg) inhibited the growth of pathogenic bacteria like *Staphylococcus aureus*, *Aeromonas veronii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Salmonellatyphi* (Thirupathy *et al.*, 2014; Inalegwu and Sodipo, 2015). The dichloromethane extracts of the root and leaf of *T. vogelii*(Family: Fabaceae) grown in

Kenya inhibit the growth of*Staphylococcus aureus, Escherichia coli* and *Fusarium phoseolida* (*Wangaetal*., 2006).*T. vogelii* and *Moringa oleifera* extracts(1g/25 ml) were used in the control of insect pests of the water melon (Alao and Adebayo, 2015). Thedichloromethane extracts from the roots and leaves of*T. vogelii* was found to possess antimicrobial activityagainst *S. aureus, E. coli* and *F.phoseolida* (Wanga *et al*., 2006). Hu *et al.,* in 2011 also studied the antimicrobial and bactereostatic activity ofethanol and aqueous extracts(2g/40 ml) from *T. vogelii* seeds on *E. coli, S. aureus* and *S. paratyphi*B. and proved the antibacterial efficacy of the plant to be significant at high doses (Hu *etal*., 2011).

2.2.2.3Antifungal activity

Three medicinal plants (*Milletia laurentii, T. vogelii, and Croton macrostachyus*) extracts (100mg/kg) traditionally used to manage infectious diseases and inhibit the growth of*Trichophyton rubrum, Trichophytonsoudanense* and *Trichophyton violaceum (*Teugwa *etal*., 2013*).*

2.2.2.4Insecticidal activities

Evaluation of insecticidal activities of *T. Vogelii*and *Azardirachta indica* A. Juss. (L) extracts(300mg/kg) against Podagricaspecies and *Zonocerus variegatus* compared to a synthetic insecticide were investigated in cultivated roselle in Ogbomoso, Nigeria which showed that they have effect on them **(**Olaniran et *al*., 2013*).T. vogelii* is used as a seed dresser (insecticide/repellent) for cereals and legumes **(**Nwude, 1997). *T. vogelii* is applied directly to treat head lice, fleas, scabies and other ectoparasites **(**Klaassen, 1996; Nwude, 1997).*T. vogelii* and *Lantana camara,* have moderate to strong bioactivity (toxicity, repellence and anti-feeding) against the golden flea beetle and hold good promise as eco-

friendly and cost effective alternatives to synthetic pesticides for field insect pest control **(**Igogo, 2013). *T. vogelii* leaf extract was found to be effective in controlling ticks, an important insect and ectoparasite (Gadzirayi *et al.,* 2010). In 2012, Kalume *et al*., reported the acaricidal activity of leaf extracts of *T. vogelii* on tick *Rhipicephalus appendiculatus* and mentioned its advantage of being economical than synthetic compounds (Kalume *et al*., 2012).

2.2.2.5Hemolytic activities

Study has revealed that the leaves of the plant *T. vogelii* Hook. f. containedsaponinswhich were responsible forits haemolytic activities against blood group O, A, B and AB (Inalegwu and Sodipo, 2013).

2.2.2.6 Anthelminticactivities

The leaf extract(50mg/kg) of *T. vogelii* was found to possess significant anthelmintic activity against *Ascaridia galli*, a parasite in chicken (Siamba *et al*., 2007). The methanol extract of *T. vogelii* produced significant reduction in the blood pressure of cats (Adaudi *et al*., 2009).

2.2.2.7 *Larvicidalactivities*

The extracts of *T. vogelii*(100mg/kg)also possess larvicidal activity and therefore can be used to control mosquitoes (Matovu and Olila,2007; Vasconcelos *et al*., 2009). *T. vogelii* leaf extract (10 mg/25 ml)exerted a stimulant effect on the GIT smooth muscles. This was demonstrated by the contraction of the ileum isolated from guinea pig hence showing the purgative property of the plant (Dzenda *et al*., 2008)

2.2.2.8. Anti-inflammatory activities

The methanolic extract(100mg/kg) of *T. vogelii* showed significant analgesic and anti-inflammatory activity in mice and rats using hot plate method and egg albumin induced oedema respectively (Adaudi *et al.*, 2009).

2.2.2.9 Antioxidant/ *Toxicities*

The ethanol ether extract(50mg/kg) of *T. vogelii* seeds showed antioxidant and free adical scavenging activity (Li *et al*., 2010).*T. vogelii* root and leaf extracts were found to be toxic to brine shrimps at doses of LC_{50}: 0.960; 0.958 µg/ml respectively (Wanga *et al*., 2006). The toxicity of *T. vogelii* was reported on mice. The signs were similar to those associated with the toxicity from rotenone. The LD_{50} of leaf extract calculated was 134.16 mg/kg (Dzenda *et al*., 2008).

Water extract of *T. vogelii* is very toxic to fish and other aquatic organisms when compared with other icthyotoxins of plant origin (Agbon, 2004).

2.2.3*Antihyperlipidemic effect of plants*

The antihyperlipidemic effect of *Tephrosia calophylla* has been studied in wistar albinorats (Mohan, 2011). The leaf extract(300mg/ml)of *Tephrosia purpurea* showed antihyperlipidemicactivity in an experimental model of diabetic rats (Pavana *et al.*, 2007). Akhtar *et al*., alsostudied *Tephrosia purpurea* for the same purpose and found a significant reduction in all theparameters (Akthar *et al*., 2011).*C. ternatea* leaves and flowers extract exhibit antihyperlipidemic effects and consequently may alleviate liver and renal damage associated with alloxan-induced diabetes mellitus in rats (Daisy *et al*., 2009). Ethanol and aqueous extract of *Thespesia populnea* exhibited significant antihyperglycemic and

antihyperlipidemic effects on alloxan- induced diabetic rats (Belhekar *et al*., 2013). *C.unshiu* shows more antihyperlipidemic effect than antidiabetic effect (Ji *et al*., 2006). Aqueous *U. dioica* extract significantly reduced the level of serum triglyceride and cholesterol (Suleiman *et al*., 2012). The hypolipidemic efficacy of the juice of the fruit of *Lagenaria siceraria* is also substantiated by the calculated cardiovascular risk factor. Juice of the fresh fruits of *Lagenaria siceraria* have the potential to cause a blood cholesterol lowering effect. The serum biochemistry changes may suggest that the juice extract has a tonic effect on the kidneys and the liver and these organs play central role in drug metabolism (Pankaj *et al*., 2011). Ethanolicextract of *E. hirta* had significantly decreased total cholesterol, triglycerides, VLDLand LDL with increase in HDL which is having a protective function for the heart (Anup *et al*., 2012).

2.2.4 Animal feed

T. vogelii, Tephrosia candida, and *Tephrosia purpurea*can also be a good addition in the diet of ruminants (Mbomi *et al*., 2011).

2.2.5 Phytochemical analysis of T. vogelii

Phytochemical analysis of the leaves extract(200mg/ml) *of T. vogelii* indicated the presence of alkaloid, tannin, saponin, cardiac glycoside, rotenone, steroids, balsam, phenol and volatile oil (Akpa *et al*, 2010; Makoshi and Arowolo, 2011; Inalegwu and Sodipo, 2013).

2.3 Hyperlipidemia

2.3.1 Definition and composition of hyperlipidemia

Hyperlipidemia is defined as an elevation of lipids in plasma (McKenney *et al.*, 2001). Hyperlipidemia is a high concentration of all the different blood fats (also called lipids) in

the blood while hypercholesterolemia is a high level of cholesterol in the blood(Maton, 1993). Hyperlipidemia is an increase (*hyper*) in the lipids(*lipi*), which are a group of fats or fat-like substances in the blood (*demia*) (Javed *etal*., 2009). Cholesteroland the triglyceridesare the two major lipids in the blood. Hyperlipidemia is also called hyperlipoproteinemia because these fatty substances travel in the blood attached to proteins (The fat-protein complexes in the blood are called lipoproteins). Hyperlipidemia is characterized by elevated serum total cholesterol, low-density lipoprotein cholesterol, very low density lipoprotein cholesterol and decreased high-density lipoprotein cholesterol levels (Javed *etal*., 2009). Elevation of one or bothof these lipids are seen in hyperlipidemia. Serum cholesterol levels above 200 mg/dL and triglyceride levelsabove 150 mg/dL are associated with atherosclerosis. It is the most common form of dyslipidemia. Hyperlipidermia is one of the greatest risk factors contributing to prevalence and severity of cardiovascular diseases.Cardiovascular disease or cardiovascular diseases is the class of diseases that involve the heart or blood vessels (arteries and veins) (Maton, 1993). While the term technically refers to any disease that affects the cardiovascular system (as used in International Statistical Classification of Diseases and Related Health Problems 10^{th} Revision: Diseases of the circulatory system). This disease includes atherosclerosis, myocardial infarction (heart attacks), cerebrovascular diseases, stroke, angina, aneurysm, congestive heart failure, peripheral vascular disease, high blood pressure and coronary artery disease. It has been noted that cholesterol along with some other types of fats cannot be dissolved in the blood. Moreover, in order to be transported to and from cells, they have to be specially carried by certain molecules called lipoproteins, which consist of an outer layer of protein with an inner core of cholesterol and triglycerides

(Kritchevsky, 1988; Dargel, 1989). In addition, the lipoproteins have been found essential for cholesterol to move around the body.

2.3.2 Lipids of biological importance

The lipids can be classified as total cholesterol (TC*)*, triglycerides, low density lipoprotein (LDL*)*,high density lipoprotien (HDL*)* and very low density lipoprotein (VLDL) cholesterol.

*2.3.2.1Total cholesterol (TC)***:** Cholesterol is an essential component of the cell membrane and starting material for the biosynthesis of bile acid, steroid hormones and vitamin D (Libby *et al*., 2000).According to guidelines of National Cholesterol Education Program (NCEP), TC concentrations below 200 mg/dL have been regarded as desirable, whereas, concentrations greater than 200 mg/dL are referred to as hyperlipidemic. However, epidemiological evidence suggests that the risk of cardiac events decreases as TC levels fall approximately to 150 mg/dL. Moreover, TC should be less than 180 mg/dL for children (Ahmed *etal*., 1998; Fryar *etal*., 2010). Hypercholesterolemia is a high level of cholesterol in the blood.Reduction of 1% cholesterol can lead to 2-3% reduction of CHD risk (Ornish and Rosner, 2005). Cholesterol in food is frequently confused with cholesterol in the blood. Foods that are high in cholesterol include fatty red meat, eggs and animal fats such as butter and cream, as well as some plant sources like coconut and palm oil. Consuming plenty of food with high levels of saturated fat and trans fatty acids along with foods high in cholesterol results in an increase of cholesterol in the blood, which increases the risk for a heart attack. The most important ones are low-density lipoprotein (LDL) known as the "bad" cholesterol which carries cholesterol to the heart and high-density lipoprotein (HDL),

which is the good blood cholesterol which carries cholesterol from heart. A high level of LDL cholesterol increases the risk of heart disease. Therefore, the lower the LDL cholesterol is, the lower the risk of heart attack and stroke will be. Higher HDL levels are better and give some protection against heart disease, whereas low HDL cholesterol puts you at higher risk for heart disease. Everybody has cholesterol present in the blood and all parts of the body. It is only when the level gets too high that the risk for heart attacks increase. Usually those with total blood cholesterol levels of 5mmol/L or higher is said to have hypercholesterolemia (Suzuki, 2006).

Fatty acids are part of all fats and oils in food and in the body. Some fatty acids promote heart health if consumed in limited amounts while others have a detrimental effect by increasing blood cholesterol levels. The detrimental fatty acids are those in high concentrations of hard animal fats, such as fat in meat, butter and cream and are known as saturated fatty acids. With the discovery that saturated fats have adverse effects on blood fats, people turned to plant oils as a safe replacement for the saturated animals fats. More recently, it has also been found that harder fats (such as brick Margarine) contain Trans fatty acids which are formed during the process of hydrogenation (hardening) of the vegetable fats.Trans fats also raise cholesterol levels and hence should be avoided. Tub (softer) margarines are manufactured in such a way that they contain almost no trans fatty acids.

Cholesterol is present in tissues and in plasma either as free cholesterol or as a storage form, combined with a long-chain fatty acid as cholesteryl ester. In plasma, both forms are transported in lipoproteins. Cholesterol is an amphipathic lipid and as such is an essential structural component of membranes and of the ousters layer of plasma lipoproteins. As a

typical product of animal metabolism, cholesterol occurs in foods of animal origin such as egg yolk, meat, liver, and brain. Plasma low-density lipoprotein (LDL) is the vehicle of uptake of cholesterol and cholesteryl ester into many tissues. Free cholesterol is removed from tissues by plasma high-density lipoprotein (HDL) and transported to the liver, where it is eliminated from the body either unchanged or after conversion to bile acids in the process known as reverse cholesterol transport. However, its chief role in pathologic processes is as a factor in the genesis of atherosclerosis of vital arteries, causing cerebrovascular, coronary, and peripheral vascular disease (Robert *et al.*, 2006).

2.3.2.2 Triglyceride (TG):Triglycerides are another type of fat that is carried in the blood by LDL. Moreover, it has been shown that excess calories, alcohol or sugar in the body get converted into triglycerides and stored in fat cells throughout the body (Ginsberg and Goldberg, 2001; Smelt, 2010).The triglyceride concentration of 110-150 mg/dL is regarded as normal, whereas, concentrations of 160-499 mg/dL are considered as high as well as concentration of 10-100mg/dl are considered very low. Moreover, concentrations of 500 mg/dL or higher are considered dangerous for the development and progression of various CVDs.

*2.3.2.3 Low density lipoprotein (LDL)***:** LDL is commonly known as the bad cholesterol, which is produced by the liver and carry cholesterol and other lipids from the liver to different areas of the body like muscles, tissues, organs and heart. The high levels of LDL indicate much more cholesterol in the blood stream than necessary and hence, increase the risk of heart disease (Ahmed *etal*., 1998; Ginsberg and Goldberg, 2001; Costet, 2010).

According to NCEP guidelines, LDL cholesterol concentrations below 100mg/dL are considered optimal, whereas concentrations in the range of 160-189 mg/dL are considered

to the higher side. However, increasing evidence supports that normal human LDL cholesterol concentration can be as low as 50 to 70 mg/dL. Moreover, it has been comprehensively seen that the risk of CVDs decreases as LDL cholesterol concentration decreases.

2.3.2.4 High density lipoprotein (HDL): HDL is commonly referred to as the good cholesterol, which is produced by the liver to carry cholesterol and other lipids from tissues back to the liver for degradation (Ginsberg and Goldberg, 2001; Ridke *etal*., 2010).

High levels of HDL cholesterol have been considered as a good indicator of a healthy heart. The concentrations of 60 mg/dL or higher have been considered as optimal, whereas, HDL concentrations below 40 mg/dL are considered as major risk factor for CVDs. However, HDL is often interpreted in the context of TC and LDL concentrations, and hence may be regarded as less significant when LDL is low (Ginsberg and Goldberg, 2001; Ridke *etal*., 2010).

*2.3.2.5 Very low density lipoprotein (VLDL)***:** VLDL is similar to LDL cholesterol in the sense that it contains mostly fat and not much protein. VLDL cholesterol is the lipoproteins that carry cholesterol from the liver to organs and tissues in the body (Sundaram and Yao, 2010). They are formed by a combination of cholesterol and triglycerides. Moreover, VLDLs are heavier than LDL, and are also associated with atherosclerosis and heart disease.

2.3.3 Classification of hyperlipidemia

Hyperlipidemia is classified into primary and secondary depending on its etiology (Costet, 2010).Hyperlipidemia (disorder) may be classified as either familial form (also called

primary) caused by specific genetic abnormalities, or acquired or non-familial form (also called secondary) when resulting from another underlying disorder that leads to alterations in plasma lipid and lipoprotein metabolism. Also, hyperlipidemia may be idiopathic, that is without known cause.

2.3.3.1 Primary hyperlipidemia: This Primary hyperlipidemia or familial hyperlipidemia (Ginsberg and Goldberg, 2001).can be further classified into five according to the Fredrickson classification which is based on the pattern of lipoproteins on electrophoresis or ultracentrifugation (Kumar *etal*., 2002). It was later adopted by the World Health Organization (WHO). Presumably, each variety of familial hyperlipidemia could arise from the action of a single gene (monogenic or Mendelian inheritance) or could reflect the interaction of several genes at many different loci (polygenic inheritance) (Joseph *etal*., 1973). This is as a result of deficiency in enzymes such as lipoprotein lipase or altered apolipoprotein C 11 responsible in triacylglycerol and cholesterol metabolism. If these two forms occur together, then it is called mixed hyperlipidemia (Baron, 2005). It does not directlyaccount for HDL, and it does not distinguish among the different genes that may bepartially responsible for some of these conditions. In the past it was a popularsystem of classification but is considered out-dated by many experts now. Following are the five types of hyperlipidemia (Kumar *etal*., 2002).

2.3.3.1.1Hyperlipoproteinemia Type-I: hyperlipoproteinemia: Type I also called primary hyperlipoproteinaemia orfamilial hyperchylomicronemia) is due to deficiency of lipoprotein lipase (LPL) oraltered apo lipoprotein C2, resulting in elevated chylomicrons, the particles thattransfer fatty acids from the digestive tract to the liver. Its occurrence is

0.1% of thepopulation. This is quite uncommon according to "Harrison's Principles of Internal Medicine" by Anthony S Fauci. (Raised cholesterol with high triglyceride levels).

2.3.3.1.2 Hyperlipoproteinemia Type-II: hyperlipoproteinemia: Type II, the most common form, is further classified intotype IIa and type IIb, which are as follows

2.3.3.1.2.1 Familial hypercholesterolemia or hyperlipoproteinemia Type-IIa: Hyperlipoproteinemia Type-IIa may be sporadic, polygenic, or truly familial as a result of mutation either in the LDL receptor gene on chromosome 19 or the Apo B gene. The familial form of this type is characterized by tendon Xanthoma, xanthelasma and premature cardiovascular disease (High cholesterol with normal triglyceride levels).

2.3.3.1.2.2 Familial combined (mixed) hyperlipidemia or hyperlipoproteinemia Type-IIb Hyperlipoproteinemia Type-IIb is caused by high VLDL levels which are due to overproduction of substrates, including triglycerides, acetyl CoA, and an increase in B-100 synthesis. They may also be caused by the decreased clearance of LDL.

2.3.3.1.3 Familial dysbetalipoproteinemia or hyperlipoproteinemia Type-III

Hyperlipoproteinemia Type-III is due to high chylomicrons and IDL (intermediate density lipoprotein). It is also known as broad beta disease or dysbetalipoproteinemia, which is mostly due to the presence of Apo E E2/E2 genotype. It is due to cholesterol-rich VLDL. This is an uncommon disorder (Raised cholesterol and triglycerides).

2.3.3.1.4 Familial hypertriglyceridemia or hyperlipoproteinemia Type-IV

Hyperlipoproteinemia Type-IV also known as hypertriglyceridemia or pure hypertriglyceridemia is due to high triglycerides. According to the NCEP (National Cholesterol Education Program) definition of high triglycerides, occurrence is about 16% of adult population (Raised triglycerides, atheroma, and raised uric acid).

2.3.3.1.5 Familial mixed hypertriglyceridemia or hyperlipoproteinemia Type-V

Hyperlipoproteinemia Type-V is very similar to type I, but have high VLDL in addition to chylomicrons. This disease has glucose intolerance and hyperuricemia (Raised triglycerides).

*2.3.3.2 Secondary/acquired hyperlipidemias***:** Secondary/acquired hyperlipidemias results from: Liver disease, biliary disease, Obesity, Hypothyroidism, Diabetes, Diet, Alcohol excess, renal disease (nephrotic syndrome), Drugs (HIV protease inhibitors, thiazide diuretics, and oral contraceptive steroids), hence called acquired hyperlipidemia (Chait and Brunzell, 1990). The most severe hyperlipidemias usually occur in patients with concurrent conditions, e.g. diabetes Mellitus with one of the primary hyperlipidemias.

2.3.4 Inducers of hyperlipidemia

2.3.4.1 High fat diet

A high-fat diet is a diet that increases the consumption of dietary fat beyond the level of an average person. There is no formal definition for a high-fat diet, but diets that are high in fat content typically derive at least 50 percent of total calories from fat nutrients. Based on a 2,000 calorie diet, this is equivalent to at least 111 g of fat. By comparison, the American Academy of Family Physicians (AAFP), states that the typical American diet gets 35 percent of its calories, or 85 g, from dietary fat (Jacobs, 2010).

Several gene-targeted mice have been effectively used to study Apo B metabolism. Expression of human Apo B in mice fed a high-fat diet resulted in a 20-fold increase in the plasma content of Apo B-containing lipoproteins and extensive atherosclerosis. Since the secretion of Apo B from hepatocytes of these mice was only slightly higher than in wild-

type mice the lipoprotein accumulation and atherosclerosis were attributed primarily to defective lipoprotein clearance (Vance *et al.*, 2002).Consumption of a high fat diet, insulin, glucose and glucocorticoids induce obese expression (Vance *et al.*, 2002). The type of fat should be considered when choosing a high-fat diet for an animal study. Many high-fat diets used in laboratory animal research contain more saturated fat such as lard, beef tallow, or coconut oil and these diets are quite capable of inducing obesity in susceptible strains. In contrast, omega-3 fatty acids have been studied for their health benefits. For example, it has been shown that of animals fed similar amounts of fat, those fed diets containing fish oil did not gain as much weight as those fed diets with more saturated fat and were more insulin sensitive. Since fatty acids can affect phenotype through a variety of mechanisms (gene expression, eicosanoid production, membrane receptor function), it is important to include information about the type and level of fat used in a study in order to allow other researchers to compare data (Angela, 2008).

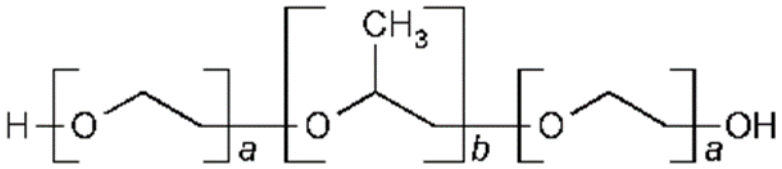
High-fat diets used in laboratory research typically contain about 32 to 60% of calories from fat. From a nutritional perspective, a human diet of 60 kcal% fat would be considered extreme. That said, diets with 60 kcal% fat are commonly used to induce obesity in rodents since animals tend to gain more weight more quickly, thereby allowing researchers to screen their compounds after a shorter period of time. It should also be noted that when studying the effects of a drug, nutraceutical, or gene mutation on obesity, it may be more difficult to prevent or reverse the effects of a very high-fat diet, when it might be possible with a diet containing a lower percentage of fat (Angela, 2008).

2.3.4.2 Poloxamer

Poloxamer 407 is a hydrophilic non-ionic surfactant belonging to the group known as poloxamers. It is a triblock copolymer consisting of a central hydrophobic block of polypropylene glycol flanked by two hydrophilic blocks of polyethylene glycol (PEG)(Megalli *etal*., 2005). The approximate length of the two PEG blocks is 101 repeat units while the approximate length of the propylene glycol block is 26 repeat units(Angela, 2008).This particular compound is also known by the BASF trade name Pluronic RF-127. Poloxamer 407 (Pluronic RF-127) has been used to induce hyperlipidemia in rats. P407 is a biocompatible, non-ionic surfactant, considered non-toxic and safe during chronic administration for long term studies (Megalli *etal*., 2005).

The following information about poloxamer are obtained:

2.3.4.2.1 Structure of poloxamer



2.3.4.2.2 Properties of poloxamer:

Poloxamer is a synthetic block copolymer of ethylene oxide and propylene oxide. It is available in several types, conforming to Table 2.1

Table 2.1*Properties of poloxamer*

Poloxamer	Physical Form	Average Molecular Weight	Weight % Oxyethylene	Unsaturation,mEq/g

Poloxamer	Physical Form	Average Molecular Weight	Weight % Oxyethylene	Unsaturation,mEq/g
124	Liquid	2090to 2360	46.7±1.9	0.020±0.008
188	Solid	7680to 9510	81.8±1.9	0.026±0.008
237	Solid	6840to 8830	72.4±1.9	0.034±0.008

It contain a suitable antioxidant (Megalli *etal*., 2005).

Poloxamers are nonionic triblock copolymers composed of a central hydrophobic chain of polyoxypropylene (polypropylene oxide)) flanked by two hydrophilic chains of polyoxyethylene (poly(ethylene oxide)). The word "poloxamer" was coined by the inventor, Irving Schmolka, who received the patent for these materials in 1973 (Costet, 2010).Poloxamers are also known by the trade names Synperonics, Pluronics and Kolliphor. Because the lengths of the polymer blocks can be customized, many different poloxamers exist that have slightly different properties. For the generic term "poloxamer", these copolymers are commonly named with the letter "P" (for poloxamer) followed by three digits, the first two digits x 100 give the approximate molecular mass of the polyoxypropylene core, and the last digit x 10 gives the percentage polyoxyethylene content (e.g., P407=Poloxamer with a polyoxypropylene molecular mass of 4,000 g/mol and a 70% polyoxyethylene content). For the Pluronictradename, coding of these

copolymers starts with a letter to define its physical form at room temperature (L = liquid, P = paste, F = flake (solid)) followed by two or three digits, The first digit (two digits in a three-digit number) in the numerical designation, multiplied by 300, indicates the approximate molecular weight of the hydrophobic; and the last digit x 10 gives the percentage polyoxyethylene content (e.g., L61 = Pluronic with a polyoxypropylene molecular mass of 1,800 g/mol and a 10% polyoxyethylene content). In the example given, poloxamer 181 (P181) = Pluronic L61.

2.3.4.2.3 Uses of poloxamer:

Because of their amphiphilic structures, the polymers have surfactant properties that make them useful in industrial applications. Among other things, they can be used to increase the water solubility of hydrophobic, oily substances or otherwise increase the miscibility of two substances with different hydrophobicities. For this reason, these polymers are commonly used in industrial applications, cosmetics, and pharmaceuticals. They have also been evaluated for various drug delivery applications and were shown to sensitize drug resistant cancers to chemotherapy (Poloxamer have also been shown to inhibit Multiple Drug Resistance (MDR) proteins and other drug efflux transporters on the surface of cancer cells; the MDR proteins are responsible for the efflux of drugs from the cells and hence increase the susceptibility of cancer cells to chemotherapeutic agents such as doxycycline) Poloxamer 188 is a complex polydisperse mixture of non-ionic macromolecules. Adverse non-IgE-mediated hypersensitivity reactions occur in some individuals following intravenous injection of poloxamer 188-based pharmaceuticals, presumably via complement activation. In bioprocess applications, pluronic is also used in cell culture

media for its cell cushioning effects because its addition leads to less stressful shear conditions for cells in reactors.

In materials science, the poloxamer P123 has recently been used in the synthesis of mesoporous materials, including SBA-15 (Angela, 2008). Poloxamer 188-mediated complement activation is an intrinsic property of these macromolecules and was independent of the degree of sample polydispersity, as opposed to other non-polymeric constituents. By quasi-elastic light scattering, interaction was established between poloxamer and lipoproteins; interestingly, poloxamer-induced rise in SC5b-9was significantly suppressed when serum HDL and LDL cholesterol levels were increased above normal to mimic two relevant clinical situations (Moghimi*et al*., 2004).

2.3.4.2.3.1 Specific Uses of Poloxamer 407:

Nash *et al*., (1996) attempted to determine the mechanism(s) of poloxamer (P)-407-induced hyperlipidemia in rats by administering a lipid-lowering drug with a known mechanism of action. The data obtained suggest that P-407 may act by stimulating the release of free fatty acids from the adipocyte for at least 24 hours after injection. Also in Zobaer *et al*., (2011) the animals (Swiss albino mice) were made hyperlipidemic by an intraperitoneal injection of 600 mg/kg body weight of poloxamer 407, followed by a 6 h fasting. The poloxamer 407 was made at a final concentration of 30% (w/w) by dissolving the powder in distilled normal saline; the solution was then kept refrigerated overnight to facilitate its dissolution.

Young *et al*., (2012) shows that rats can be hyperlipidemically induced by poloxamer 407 (HL rats). The total area under the plasma concentration–time curve (AUC) of carbamazepine (CBZ) in HL rats after intravenous administration was significantly greater than that in controls due to their slower non-renal clearance (CLNR). Their findings have potential therapeutic implications assuming that the HL rat model qualitatively reflects similar changes in patients with hyperlipidemia. According to Hetal and Dion (2013) the induction of hyperlipidemia using poloxamer 407 (P407) is gaining use for studying the effect of the condition on drug pharmacokinetics. Although a single intraperitoneal dose of P407 causes a rapid onset of hyperlipidemia, the initial lipid concentrations are much higher seen in humans. The hyperlipidemia is also reversible in nature. Single 0.5 and 1 g/kg doses of P407 were injected into rats followed by blood collection at various times for up to 12 days. The maximum increase in lipids was observed at 36 h, with most lipids remaining elevated for up to 180mg/dl, although for the 1 g/kg dose triglyceride concentrations had still not quite returned to baseline by 12 days post dose (Hetal and Dion, 2013) concluded in their investigation that: depending on study aims, for the use of the model it may be beneficial to perform single-dose assessments at time points later than 36 h when the lipoprotein concentrations will be more similar to those seen in patient with hyperlipidemia. P407 increases serum lipoproteins via its actions at various levels in lipid metabolism, largely by inhibiting lipoprotein lipase, which facilitates the hydrolysis of triglycerides. P407 also causes indirect stimulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase which is involved in cholesterol biosynthesis (Hetal and Dion, 2013).

Hetal and Dion (2013) explained further that the hyperlipidemia induced by a single dose of P407 is reversible in nature; within 2-3 days after injection it is visually evident that hyperlipidemia subsides, as the plasma/serum gradually changes from a milky white to a normal clear appearance. The plasma concentrations of lipids have been followed after dosing of rats with a single dose of 0.4 g/kg. Johnston and Palmer, (1993) earlier in their investigation found that: One 300 mg i.p. injection of the nonionic surfactant poloxamer 407 (Pluronic F-127) produces a significant increase above control of both circulating cholesterol and triglyceride (TG) concentrations. Concentrations of total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) were observed to fall after 12-24 h of dose administration. However, low density lipoprotein cholesterol (LDL-C) was still rising and the other serum lipids were still substantially higher than baseline levels at 48h from the time of P407 dosing.Study was conducted to determine the effect of poloxamer 407 (P-407) on the capacity to hydrolyze circulating TG by lipoprotein lipase (LPL) in an attempt to determine the mechanism of action of P-407. The concentration of TG in the rat following a single 300 mg i.p. injection of P-407 was marked, increasing from 84 +/- 10 to 3175 +/- 322 mg/dL at 24 hr. The maximal rate of TG accumulation (5.74 mg/dL/min) in the plasma of P-407-injected rats occurred between 2 and 4 hours.post-injection Hetal and Dion (2013).

2.3.4.3 Others

Cyclosporin A (CsA) is an immunosuppressant drug widely used in organ transplant recipients and patients with auto-immune disorders. Long-term treatment with CsA is associated with hyperlipidemia and an increased risk of atherosclerosis. In mice, CsA increased the rate of hepatic VLDL, LDL, TC and decreased HDL secretion *in vivo* (Wu *et*

al., 1999). Butter was used as the hyperlipidemic inducer in rats (Sowmya and Ananthi,2011). The evaluation of antihyperlipidemic activity of ethanolic extact of *Glycosmis pentaphylla* was done using Triton X 100 and High Fat Diet induced hyperlipidemia models in Wistar albino rats (Syed *et al*., 2015).Triton WR1339 (Tyloxapol) has been used by several studies to induce hypercholesterolemia in animals (Luiz*et al*., 2011).Diet containing 25 % soybean oil, 1.0 % cholesterol, 13 % fiber and 4,538.4 Kcal/Kg increase LDL-cholesterol and decrease HDL in rats (Sheyla *et al*., 2005). A high calorie diet, standardized by Estadella *et al*., (2004), containing 19% protein, 47% carbohydrate, 16% lipids, 3% cellulose, 5% vitamins and 19.38 kcal g-1 minerals. The ingredients were ground and mixed in pellet form at the following ratio: 15 g of normocaloric feed Nuvilab (3.78 kcal g-1), 10 g roasted peanuts (5.95 kcal g-1), 10 g milk chocolate (5.4 kcal g-1) and 5 g of corn starch biscuits (4.25 kcal g-1) increase serum LDL-cholesterol and decrease HDL in rats.Isoproterenol, a synthetic catecholamine and β-adrenergic agonist has reported to cause oxidative stress in the myocardium, resulting in infarct-like necrosis of heart muscle (Wexlerand Greenberg, 1978). The pathophysiological changes following isoproterenol administration were compared to human myocardial infarction (Wexlerand Greenberg, 1978). Isoproterenol increase cholesterol, triglycerides, LDL and VLDL with a decrease in HDL cholesterol in albino rats (Ganesh and Juvekar, 2006).Fructose has been reported to induce hypertriglyceridemia associated with insulin resistance, hyperinsulinemia and hypertension (Dai and McNeill, 1995).Twenty five percent (25%) of D-fructose in drinking water was used as an inducing agent for hyperlipidemia inexperimental rats. Diet high in fructose induces insulin resistance (IR) in rats (Thorburn *et al*., 1989), hamsters (Wexlerand Greenberg, 1978) and dogs (Martinez *et*

al., 1994). Investigators have produced IR by administering fructose in drinking water (5-10%) or by feeding a diet in which fructose contributes to more than 50% of total calories. Rats develop IR, hyperlipidemia, and hypertension as early as 2 weeks of fructose diet initiation (Dai and McNeill, 1995). Atherogenic diet containing 0.2% cholesterol and 0.6% groundnut oil in standardhas been used to induce hypercholesterolemia in rats (Wexlerand Greenberg, 1978). Lipofundin 20% induces hyperlipidemia, which promotes an oxidative stress state in Sprague Dawley rats (DelgadoRoche *et al*., 2012). Lipofundin is a lipid emulsion used in parenteral nutrition.

2.3.5 Causes/ risk factors of hyperlipidemia

The majority of cardiovascular disease (CVD) is caused by risk factors that can be controlled,

treated or modified, such as high blood pressure, cholesterol, overweight/obesity, tobacco use,lack of physical activity and diabetes. However, there are also some major CVD risk factors thatcannot be controlled, such as age, gender and family history.

2.3.5.1 Lifestyle, overweight and obesity

Mostly hyperlipidemia is caused by lifestyle habits or treatable medical conditions (Fuller *etal*., 1980). Lifestyle habits include obesity which is one of the common chronic disorders of cholesterol and carbohydrate metabolism is characterized by excess fat deposition in adipose tissue, liver, heart, skeletal muscle, and pancreatic islet (World Health Organization, 1997; Ahima, 2006).Sedentary lifestyle and excessive body weight may be very important cause of cardiovascular diseases. Cardiovascular disease along with diabetes is the most characteristic condition in developed countries. Sedentary life and diet high in saturated fats plays a key role in development of both diseases (Fuller *etal*., 1980). It is

referred to one where minimal physical activity (exercise) is present. There are no health benefits because of this lack of regular physical activity. People with sedentary lifestyle are more prone to the diseases, when compared to those who exercise thrice a week (Fuller *et* al., 1980). Overweight is considered when body mass index (BMI) ≥25 kg/m^2 and morbidly obese (BMI≥30) (Kuczmarski *etal*.,1991; Wickelgren, 1998). Obesity has a negative influence on blood pressure and cholesterol, and may lead to diabetes. Body Mass Index (BMI): This index reflects a person's weight terms of his or her height. This is defined as the weight in kilogram divided by height in meters square (kg/m^2). A person is obsese when his or her BMI is 30kg/m^2 or higher. A person is overweight when his or her BMI is 25 kg/m^2. BMI < 18.5 is underweight, BMI between18.5-24.9 is normal weight and BMI between 25.0 29.9 is overweight.

2.3.5.2 Age

Age is an important risk factor in developing cardiovascular diseases. It is estimated that 87 percent of people who die of coronary heart disease are 60 and older (Mackay, *etal*., 2004). At the sametimes, the risk of stroke doubles every decade after age 55 (Mackay, *etal*., 2004). Blood cholesterol levels increase with age, especially in people who follow a typical western lifestyle (Norman *etal*., 2006). Multiple explanations have been proposed to explain why age increases the risk of cardiovascular diseases. One of them is related to serum cholesterol level (Jousilahti *etal*., 1999). In most populations, the serum total cholesterol level increases as age increases. In men, serum total cholesterol level decreases around age 45 to 50 years. In women, the increase continuous sharply until age 60 to 65 years (Jousilahti *etal*., 1999). Aging is also associated with changes in the mechanical and structural properties of the vascular wall, which leads to the loss of arterial elasticity and

reduced arterial compliance and may subsequently lead to coronary artery disease (Jani and Rajkumar, 2006). It is a major global public health issue and increasing prevalence across all age-groups (World Health Organization Obesity, 1998).

2.3.5.3 Gender

Men are at greater risk of heart disease than pre-menopausal women, however once past menopause, a woman risk is similar to a man's (Jani and Rajkumar, 2006). Among middle-aged people, coronary heart disease is 2 to 5 times more common in men than women (Jousilahti *etal*., 1999). In a study done by the world Health organisation, gender contributes to approximately 40% of the variation in the sex ratios of coronary heart disease mortality (Jackson *etal*., 1999). Another study reports similar results that gender difference explains nearly half of the risk associated with cardiovascular disease and one of the proposed explanations for the gender difference in cardiovascular disease is hormonal difference (Jousilahti *etal*., 1999). In women, oestrogen is the predominant sex hormone. Oestrogen may have protective effects through glucose metabolism and haemostatic system, and it may have a direct effect on improving endothelia cell function. The production of oestrogen decreases after menopause, and may change the female lipid metabolism toward a more atherogenic form by decreasing the HDL cholesterol level and increasing LDL, and total cholesterol levels (Jousilahti *etal*., 1999). Women who have experienced early menopause, either naturally or because they have had a hysterectomy, are twice as likely to develop heart disease as women of the age group who have not yet gone through menopause(Norman *etal*., 2006). Among men and women, there are differences in body weight, height, body fat distribution, heart rate, stroke volume and arterial compliance (Jani and Rajkumar, 2006). In the very elderly, age related large artery pulsatility and

stiffness is more pronounced in women (Jani and Rajkumar, 2006). This may be caused by the smaller body size and arterial dimensions independent of menopause (Jani and Rajkumar, 2006). It is a major global public health issue and increasing prevalence across all sexes (World Health Organization Obesity, 1998). One has a greater chance of developing hyperlipidemia if he/she is a man older than age 45 or a woman older than age 55.

2.3.5.4 Stress

Research indicates that there is a relationship between the risk of developing coronary heart disease and stress. This is because stress releases certain chemicals, adrenalins which can increase heart rate and raise blood pressure as well as send blood sugar out of control (Guzik *etal*., 2002). Stress also contributes indirectly to CVD, as people under stress may smoke and drink more than those who lead stress-free lives (Gramza and Korozak, 2005).

2.3.5.5 Air pollution

Air pollution in the Netherlands is an issue that negatively influences human health and the environment. Continuously, there is much research on the impact of air pollutant particles such as particulate matter, to improve current insights into the issue. Particulate matter is a complex mixture of particles of several sizes and chemical compositions. The main components of particulate matter are inorganic secondary particles, carbon, sea salt, metal-, silica oxides and water. Depending on the aerodynamic diameter of the particles, the term PM_{10} is used to indicate particles with a diameter up to 10 μm and $PM_{2.5}$ for particles with a diameter up to 2.5 μm. Within the $PM_{2.5}$ category there is a distinction between *fine* ($\leq$ 2.5 μm) and *ultra fine* (<0.1 μm) particles. The largest particles, are the coarse fractions that are

mechanically produced by the break-up of larger solid particles. These particles include wind-blown dust from agricultural processes, soil, unpaved roads or mining operations. Health studies show that there are significant adverse events due to exposure to $PM_{2.5}$(Mathijsen *et al.,* 2007). The range of health effects due to exposure to fine particulate matter is broad, nonetheless these are predominantly to the respiratory and cardiovascular systems, causing an increase of cardiovascular and respiratory diseases and lung cancer (McDonnell *et al.,* 2000; Pope *et al.,* 2002). Moreover, increased $PM_{2.5}$ concentrations increase the risk of emergency hospital admissions for cardiovascular and respiratory causes. Nonetheless, the causal 20 factors of the adverse effects due to exposure to $PM_{2.5}$ are difficult to disentangle since $PM_{2.5}$ contains many diverse sizes of several pollutant particles and have not yet been identified (Buringh and Opperhuizen,2002). One of the hypotheses considered on PM's mechanisms of action is the oxidative potential of the particles or specific components. Several *in vitro* studies with collected particle fractions indicate that particles with a higher oxidative potential have a greater ability to deplete antioxidant defences. Uncertainty remains concerning the cardiovascular toxicity of specific sources, especially traffic as one of the major contributors to urban PM and to the mechanisms by which the effecton the cardiovascular system is mediated.Particulate matter formed by the first mechanism is referred to as primary and PM formed by the second mechanism is referred to as secondary.

Particulate matter (PM) has been studied for their short and long term exposure effects on cardiovascular disease. Currently, $PM_{2.5}$is the major focus, in which gradients are used to determined CVD risk. For every $10\mu g/m^3$ of $PM_{2.5}$long – term exposure, there is an

estimated 8 -18% CVD mortality risk (Khallaf, 2011). Women had a higher relative risk (RR) (1.42) for $PM_{2.5}$ induced coronary artery disease than men (0.90) (Khallaf, 2011). Overall long-term PM exposure increased rate of atherosclerosis and inflammation. In regards to short-term exposure (2 hours), every 25 μg/m^2 of $PM_{2.5}$resulted in a 48% increase of CVD mortality risk (Massimo and Mannuccio, 2011). Additionally, after only 5 days of exposure, a rise in systolic (2.8mmHg) and diastolic (2.7mmHg) blood occurred for every 10.5 μg/m^3 of $PM_{2.5}$(Massimo and Mannuccio, 2011). Other research has complicated $PM_{2.5}$ in irregular heart rhyhm, reduced heart rate variability (decreased vagal tone), and most notably heart failure (Massimo and mannuccio, 2011). $PM_{2.5}$ is also linked to carotid artery thickening and increased risk of acute myocardial infarction (Massimo and Mannuccio, 2011).Exposure to PM2.5 is extremely harmful to the circulatory system and its action appears to be associated with platelet aggregation that could promote increased risk to acute thrombosisformation after exposure to the particles. The mechanisms responsible for the activation of platelets and fibrinogen levels are not yet fully elucidated, but appear to be related to the releaseof cytokines such as interleukins (Miraglia, 2014).

2.3.5.6 Others

Most diseases thatmay result in hyperlipidemia are diabetes, kidney disease, pregnancy, and an under active thyroid gland. The cause of hyperlipidemia may be genetic if a patient has a normal body weight and other members of his/her family have hyperlipidemia. If a close relative had early heart disease, there is also an increased risk of this disease (Massimo and Mannuccio, 2011). Common secondary causes of hypercholesterolemia are hypothyroidism, pregnancy, and kidney failure. Common secondary causes of hypertriglyceridemia are diabetes, excess alcohol intake, obesity, and certain prescription

medications (Harinder and Karen, 2001).An elevation of plasma lipids may be caused by a primary genetic defect or secondary to diet, drugs or diseases.

2.3.6 Symptoms and diagnoses of hyperlipidemia

Hyperlipidemia usually has no noticeable symptoms and tends to be discovered during routine examination or evaluation for atherosclerotic cardiovascular disease (Grundy *etal*., 1998; Bhatnagar *etal*., 2008). Xanthoma, xanthelasma of eyelid , chest pain, abdominal pain, enlarged spleen, liver enlarged, high cholesterol or triglyceride levels, heart attacks, higher rate of obesity and glucose intolerance, pimple like lesions across body, atheromatous plaques in the arteries, arcus senilis, xanthomata are some of the symptoms. For diagnosis of hyperlipidemia,levels of total cholesterol, low density lipoprotein cholesterol, high densitylipoprotein cholesterol, and triglycerides are measured in a blood sample. It is important to note that the lipid profile should be measured in all adults 20 years and older, and the measurement should be repeated after every 5 years. Food or beverages may increase triglyceride levels temporarily, so people must fast at least 12 hours before giving their blood samples. Special blood tests are carried out to identify the specific disorder when lipid levels in the blood are very high. Specific disorders may include several hereditary disorders, which produce different lipid abnormalities and have different risks (Harinder and Karen, 2001).

2.3.7 Pathophysiology of hyperlipidemia

The pathophysiology of hyperlipidmeia can be studied under two headings, i.e., primary hyperlipidemia and secondary hyperlipidemia. The pathophysiology of primary hyperlipidemia is that involves the idiopathic hyperchylomicronemia defect in lipid metabolism which leads to hypertriglyceridemia and hyperchylomicronemia and which is

caused by a defect in lipoprotein lipase activity or the absence of the surface apoprotein CII (Marshall, 1992). Further, in primary hyperlipidemia, the LDL cholesterol is high this is supported by the results obtained in various studies which showed that idiopathic hypercholesterolemia occur in many families of doberman pinschers and rottweilers (Marshall, 1992; American Heart Association, 2005). Moreover, hyperchylomicronemia in cats with autosomal recessive defect in lipoprotein lipase (LPL) activity showed the occurrence of primary hyperlipidemia (Castilla-Guerra *et al*., 2009). In secondary hyperlipidemia, the postprandial absorption of chylomicrons from the gastrointestinal tract occurs 30-60 min after ingestion of a meal containing fat that may increase serum triglycerides for 3-10 hours (Bennett, 1995). The diabetes mellitus patients have been noted to possess low LPL activity which further caused high synthesis of VLDL cholesterol by the liver ultimately leading to hyperlipidemia. Moreover, hypothyroidism-induced low LPL activity and lipolytic activity has been noted to reduce hepatic degradation of cholesterol to bile acids. Furthermore, hyperadrenocorticism increased the synthesis of VLDL by the liver causing both hypercholesterolemia and hypertriglyceridemia (Bennett, 1995; Baron, 2005). In liver, disease hypercholesterolemia has been noted to be caused by reduced excretion of cholesterol in the bile. Furthermore, in nephrotic syndrome, the common synthetic pathway for albumin and cholesterol causes low oncotic pressure ultimately leading to enhanced cholesterol synthesis (Stone, 1994; Castilla-Guerra *et al.*, 2009).

2.3.8 Treatment of hyperlipidemia

Presently, medications from five major classes of drugs have been reported to treat people with detrimental lipid levels include statins, nicotinic acid derivatives, fibric acid derivatives, bile acid binding resins and cholesterol absorption inhibitors (Rosuvastatin, 2003; Braamskamp *et al*., 2012).

2.3.8.1 Statins

Statins, or HMG CoA reductase inhibitors, are among the most commonly prescribed drugs in the world today. These medications have been repeatedly shown to reduce low density lipoprotein cholesterol (LDL-C) by19-60% depending on the statin and dose used (Law *et al*., 2003; Wilt *et al.*, 2004). In patients with existing coronary artery disease (CAD), the drugs decrease TG, and increase HDL. Statins have been shown to decrease incidence of non-fatal myocardial infarction, incidence of stroke, mortality from CAD (Law *et al*., 2003; Wilt *et al.*, 2004). These same benefits have also been found in patients without CAD but with cardiovascular risk factors including diabetes (Horn, 1997). For this reason, statins are considered to be first line in the management of hyperlipidemia in patients who, according to the Framingham risk calculator, are at high risk for coronary artery disease as well as those at medium or low risk who have failed to meet lipid targets with lifestyle modification (Horn, 1997).

Statins, the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductive inhibitors, are the medications that have been noted to inhibit the rate at which cholesterol is formed in the body. Moreover, statins have also been reported to help draw cholesterol to the liver for excretion (Jones, 1998). The potent members of statins class of drugs include atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin (Davidson, 2001; Koh *et al*., 2008). Alcohol and grapefruit juice should be avoided when taking statins as they may

interact with the medication. Statins need to be taken with the evening meal or at bedtime. However, statins have been associated with most common side effects like stomach upset, nausea, vomiting, headache and dizziness. Statins can also be associated with mild liver enzyme elevations, GI disturbances, headaches, myalgias, and rash. It is important not to dismiss complaints of muscle aches in youth taking statins as it may indicate a rare side effect, rhabdomyolysis (Jones, 1998).

2.3.8.1.1 Adverse effects and statin intolerance

Despite the significant effect of these drugs on mortality and morbidity, statins, like all drugs, have adverse effects. However, the HMG CoA reductase inhibitors generally have a favorable side-effect profile (Davidson, 2001; Koh *et al*., 2008). The most common adverse effects and the primary contributors to statin-intolerance are muscle symptoms and creatine kinase (CK) elevations (Bennett, 1995; Baron, 2005). There are many definitions in the literature regarding the spectrum of statin-induced muscle symptoms. One commonly used set of definitions, from the National Lipid Association (NLA).Statin Safety Assessment Task Force, is as follows: (1) myalgia: muscle pain or soreness, (2) myopathy: myalgias, weakness or cramps and CK >10x the upper limit of normal, and (3) rhabdomyolysis: CK >10 000 IU/L or CK >10X the upper limit of normal plus elevation of serum creatinine or need for intravenous hydration (Jones, 1998). The rate of statin-induced myalgia in observational studies is 10-15% while the rate of myopathy is 0.1-0.2% (Davidson, 2001; Koh *et al*., 2008). Meanwhile, rhabdomyolysis is even less common and has been seen mainly when statins have been used in combination with fibrates or cyclosporine (Davidson, 2001; Koh *et al*., 2008). In the case of myopathy or rhabdomyolysis, the NLA task force recommends discontinuing the statin. Restarting the statin after these events is

controversial; nevertheless, there is a general consensus that the clinician must weigh the risks and benefits before making this decision (Jones, 1998). However, most patients on a statin presenting with muscle symptoms will fit the definition of myalgia. If these myalgias are intolerable, the NLA recommends discontinuing the statin. Meanwhile, if the myalgias are tolerable, the statin should be continued at the same or lower dose or a statin holiday may be given (Bennett, 1995; Baron, 2005). When patients have recurrent myalgias after taking a reduced dose of their statin or after restarting their statin subsequent to a drug holiday, alternative approaches to lipid management are required. This presents a challenge to physicians managing lipids in patients with or at risk for vascular disease because non-statin lipid lowering medications have not been shown to have the same robust effect on morbidity and mortality as statins and present their own set of adverse effects (Harinder and Karen, 2001). Alternative statin regimens aimed at those who have previously been intolerant to the medications have been proposed (Jones, 1998).

2.3.8.1.2 Atorvastatin

Atorvastatin is a drug that lowers blood cholesterol: a drug that blocks the biosynthesis of cholesterol, reducing the level present in the blood, and is effective in reducing heart attacks and strokes(Jones, 1998).

Atorvastatin is a synthetic hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitor. In dosages of 10 to 80 mg/day, atorvastatin reduces levels of total cholesterol, low-density lipoprotein (LDL)-cholesterol, triglyceride and very low-density lipoprotein (VLDL)-cholesterol and increases high-density lipoprotein (HDL)-cholesterol in patients with a wide variety of dyslipidemias (Harinder and Karen, 2001).

Pharmacodynamic Properties:

Atorvastatin reduces plasma levels of total cholesterol, LDL-cholesterol, very low-density lipoprotein (VLDL)-cholesterol, triglycerides and apolipoprotein B. The greater efficacy of atorvastatin than other currently available HMG-CoA reductase inhibitors in reducing total cholesterol and LDL-cholesterol levels is believed to result from a prolonged duration of HMG-CoA reductase inhibition rather than the degree of inhibition. Like other members of its class, atorvastatin inhibits hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase in vivo and in vitro, and impedes the formation of mevalonic acid, which is the rate-limiting step in the biosynthesis of cholesterol. The reduction in intracellular cholesterol increases the number of low-density lipoprotein (LDL) receptors, thus increasing the clearance of LDL-cholesterol from plasma.

In addition, the marked reductions in triglyceride levels with atorvastatin result mainly from decreases in VLDL production, caused in part by inhibition of cholesterol synthesis. The increase in number of LDL receptors, combined with the decrease in LDL particles available to bind to these receptors, may enhance the binding of VLDL particles, thus reducing triglyceride levels (Harinder and Karen, 2001).

There are some evidence that atorvastatin, like other drugs of its class, may have beneficial pharmacodynamic effects in addition to lipid-lowering in patients with atherosclerosis and CHD. Atorvastatin improved endothelial function in patients with hypercholesterolemia or diabetes mellitus. As well, atorvastatin possibly plays a role in the stabilisation of atherosclerotic plaques by reducing the accumulation of inflammatory cells within them. The drug reduced the activation in vitro of nuclear factor Kappa-B, an inducer of chemokines involved in the inflammation in the atheromatous plaque. In studies of other effects, atorvastatin abolished macrophage infiltration in the arterial neointimal lesions in

addition to reducing atheromatous lesion size in a rabbit model of atherosclerosis, and inhibited smooth muscle cell migration and proliferation in both *in vitro* and *in vivo* studies(Koh *et al*., 2008).

Treatment with atorvastatin has been shown to reduce spontaneous and ADP-and epinephrine-induced platelet aggregation, probably because of its effect on intraplatelet NO metabolism through an increase in intraplatelet NO synthase activity. Atorvastatin also reduced the enhanced susceptibility of LDL to oxidation, decreased cholesterol accumulation in macrophages and improved red cell deformability.

Most data indicate the drug has positive or negligible effects on non-lipid risk factors for CHD. The drug generally had no appreciable effect on fibrinogen levels, and most data from randomized trials demonstrated a possibly dose-dependent reduction in C-reactive protein levels. The drug generally had no effect on lipoprotein (a) levels in dosages up to 40 mg/day, but the largest such clinical trial (the ASAP study) showed a significant reduction in this parameter after 2 years' therapy with atorvastatin 80 mg/day(Jones, 1998).

Pharmacokinetic Properties:

About 30% of an oral dose of atorvastatin is absorbed and undergoes extensive first-pass metabolism (Harinder and Karen, 2001). The drug has a bioavailability of about 14% and is >98% protein bound in the plasma. No significant changes in area under the plasma concentration-time curve or elimination half-life were observed with the administration of atorvastatin 30 minutes after food intake, although the rate of absorption was reduced(Davidson, 2001; Koh *et al*., 2008).

The single-dose pharmacokinetic parameters of atorvastatin are linear. After single doses of atorvastatin 10, 20 or 40mg in healthy male volunteers, time to reach peak plasma concentration was 0.6 to 0.9 hours (Jones, 1998). The pharmacological response (lipid-lowering action) is more accurately predicted by the dose administered than the plasma drug concentrations.

Metabolism of atorvastatin by cytochrome P450 (CYP) 3A4 produces ortho-and para-hydroxylated derivatives and various β-oxidation products. Seventy (70) % of the HMG-CoA reductase inhibitory activity associated with atorvastatin has been attributed to its active ortho- and para-hydroxylated metabolites, which are equipotent to the parent drug.

The peak plasma concentration of atorvastatin is significantly increased in patients with hepatic failure and dosage needs to be reduced in such patients. However, renal impairment has no significant effect on the pharmacokinetic parameters of atorvastatin.

Clinically significant interactions of atorvastatin are likely to occur with its concomitant use with other drugs metabolized by CYP 3A4 including erythromycin, itraconazole, ethinyl estradiol, fusidic acid and cyclosporin (Kumar *etal*., 2002).

Therapeutic Efficacy:

The lipid-lowering effects of atorvastatin have been investigated in patients with various types of dyslipidemia.

Primary hypercholesterolemia: The lipid-lowering efficacy of atorvastatin in patients with primary hypercholesterolemia is well established. The drug consistently reduces total and LDL-cholesterol levels in a nonlinear dose-dependent manner, with atorvastatin (10 to 80 mg/day) producing reductions in serum LDL-cholesterol levels of about 35 to 60% in

various placebo-controlled and non-comparative trials. Atorvastatin 10 to 80 mg/day reduced triglyceride levels by 17 to 45% and Apo lipoprotein B levels by 17 to 50% (Horn, 1997). In target US National Cholesterol Education Program (NCEP), LDL-cholesterol levels (<4.1 mmol/L) were achieved in 91 and 100% of patients with low CHD risk receiving 10 and 20 mg/day of atorvastatin, respectively in a placebo-controlled study. In the high CHD risk group, 27, 40, 64 and 82% of patients receiving atorvastatin 10, 20, 40 and 80mg, respectively, reached their target serum LDL-cholesterol levels (≤2.6 mmol/L) (Castilla-Guerra *et al*., 2009).

Atorvastatin was more efficacious in lowering serum levels of LDL-cholesterol, total cholesterol and triglycerides than milligram equivalent doses of other currently available HMG-CoA reductase inhibitors in patients with hypercholesterolemia (Harinder and Karen, 2001). In large double-blind 1-year trials, reductions in total cholesterol, LDL-cholesterol, Apo lipoprotein B and triglyceride levels were significantly greater with atorvastatin (10 to 20 mg/day) than with lovastatin (20 to 40 mg/day), pravastatin (20 to 40 mg/day) or simvastatin (10 to 20 mg/day) (Horn, 1997).

Mixed hyperlipidaemia: In patients with mixed hyperlipidemia, atorvastatin (10 to 20 mg/day) produced greater reductions in serum LDL-cholesterol and total cholesterol but lesser reductions in serum triglyceride levels than fenofibrate (200 or 300 mg/day), bezafibrate (400 mg/day) and nicotinic acid (3 g/day). The increase in HDL-cholesterol was less than that with fenofibrate and nicotinic acid and similar to that with bezafibrate (Castilla-Guerra *et al*., 2009).

Atorvastatin 10 mg/day was more efficacious in reducing serum LDL-cholesterol and triglyceride levels than simvastatin 10 mg/day in a well-designed, 6-week study involving 1378 evaluable patients with mixed dyslipidemia (the ASSET trial), and then other comparator HMG-CoA reductase inhibitors in a smaller trial (Harinder and Karen, 2001).

Type 2 diabetes: The efficacy of atorvastatin in lowering serum LDL-cholesterol levels is similar in patients with type 2 diabetes mellitus and those without the condition. Atorvastatin (10 mg/day) produced a greater reduction in serum LDL-cholesterol levels than simvastatin (10 mg/day), pravastatin (20 mg/day) or lovastatin (20 mg/day) in patients with type 2 diabetes mellitus after 6 months, and the drug was superior to simvastatin after 54 weeks in the ASSET trial. More patients given atorvastatin (80 mg/day) than (10 mg/day) achieved target NCEP LDL-cholesterol goals in the DALI study (Horn, 1997).

Atorvastatin 80 mg/day significantly reduced total cholesterol and LDL-cholesterol levels in small numbers of patients with homozygous familial hypercholesterolemia treated for 2 months (Castilla-Guerra *et al*., 2009).

Aggressive therapy in patients with coronary heart disease.

Atorvastatin is generally more efficacious than the other HMG-CoA reductase inhibitors in achieving the stricter serum LDL-cholesterol target levels in patients with established CHD, in terms of the percentage of patients achieving the targets on monotherapy with these drugs as well as the proportion of patients requiring upward dose titration or a combination with other lipid-lowering agents. This has led to investigation of whether more aggressive therapy can provide clinical benefits.

Aggressive reduction of serum LDL-cholesterol to 1.9 mmol/L, well below the recommended target, with atorvastatin 80 mg/day for 16 weeks in patients with unstable angina or non-Q-wave myocardial infarction significantly reduced the incidence of the combined primary end-point ($p = 0.048$ vs placebo) and the secondary end-point of recurrent ischaemic events requiring rehospitalisation ($p = 0.02$) in the large ($n = 3086$) randomised, double-blind, placebo-controlled MIRACL trial. There were no significant differences in other secondary end-points (death, myocardial infarction and cardiac arrest).

As well, the results of the AVERT trial showed that aggressive lipid-lowering therapy with atorvastatin 80 mg/day for 18 months was at least as effective as coronary angioplasty and usual care in reducing the incidence of ischemic events in low-risk patients with stable CHD(Kumar *etal*., 2002).

Tolerability:

Atorvastatin has been well tolerated in long-term clinical trials. In placebo-controlled studies, the incidence of adverse events (18%) in 1122 patients receiving atorvastatin up to 80 mg/day was similar to that in patients receiving placebo (18%; $n = 270$) (Harinder and Karen, 2001). No dose-related increase in adverse events was observed in these studies. Overall, the most frequently reported adverse events were constipation, flatulence, dyspepsia, abdominal pain, headache and myalgia. Adverse events reported with atorvastatin have been mild and transient (Kumar *etal*., 2002).

In general, the adverse event profile for atorvastatin was similar to that observed with other HMG-CoA reductase inhibitors. Mild hepatic involvement in the form of asymptomatic elevations in serum transaminase levels has been reported during treatment with

atorvastatin in 0.7% of patients and was responsible for discontinuation of atorvastatin in 0.3%. The incidence of persistent elevation of transaminase levels was higher in patients receiving atorvastatin in doses of 80 mg/day (2.3%) than those receiving lower doses (up to 0.6%).

The incidence of myalgia with the use of atorvastatin (1%) has been found similar to that with placebo (1%) and other HMG-CoA reductase inhibitors (2%). Although isolated asymptomatic elevation of creatine phosphokinase (CPK) has been observed in patients receiving atorvastatin, persistent elevation of CPK (>10 times elevation on 2 consecutive occasions) along with muscle pain, tenderness or weakness has not so far been reported. Case reports of rhabdomyolysis are rare with atorvastatin use, most occurring with concomitant use with other drugs such as cyclosporin, fusidic acid and gemfibrozil (Kumar *etal*., 2002).

Concomitant use of atorvastatin with cyclosporin, nicotinic acid, fibrates, and erythromycin or azole antifungals is likely to increase the risk of adverse events such as myopathy and rhabdomyolysis (Harinder and Karen 2001).

2.3.8.2 Niacin

The second class of agents that have been well reported for the treatment of hyperlipidemia is niacin that has diverse actions affecting cholesterol formation. The chief effect of niacin and its derivatives involves the decreased production of triglycerides in the body. Further, nicotinic acid has also been noted to raise HDL cholesterol levels via unknown mechanisms (Guyton *et al*., 2008). Niacin is perhaps the most potent of these drugs and has been shown to raise high-density lipoprotein cholesterol (HDL-C) and lower LDL-C as well as, in older

studies, to reduce cardiovascular events and mortality (Horn, 1997). However, the common side effects of niacin drugs include flushing, hot flashes, itching, headache, cutaneous and gastrointestinal upset, have limited its use (McCullough *et al*., 2011). Recently, there has been renewed interest in niacin, including ongoing trials evaluating mortality outcomes and investigation into attenuation of these side-effects (McCullough *et al*., 2011).

2.3.8.3 Fabric acid derivatives

The third potent class of agents for the treatment of hyperlipidemia is fabric acid derivatives which have been thought to reduce the formation and increase the breakdown of cholesterol and triglycerides and increase HDL-C levels while having a smaller effect in reducing LDL-C levels in the body (Saha *et al*., 2007; Abourbih *et al*., 2009), accounting for their hypolipidemic potential (McCullough *et al*., 2011). Common side effects of this class of drugs are heartburn, gastrointestinal, and stomach pain which have been noted to decrease over time while myalgia occurred at the same rate as in the placebo groups. The potent members of fibrates are clofibrate, fenofibrate and gemfibrozil (McCullough *et al*., 2011; Saurav *et al.*, 2012). In terms of outcomes, two recent meta-analyses found that while currently available fibrates significantly reduce the odds of non-fatal myocardial infarction (MI), they had no effect on cardiovascular mortality, rate of stroke, or all-cause mortality (Saha *et al*., 2007; Abourbih *et al*., 2009).

2.3.8.4 Bile acid binding resins

Another class of agents for the treatment of hyperlipidemia involves bile acid binding resins, which are regarded as the medications that bind with bile acids, preventing the intestine from recycling those (Kobayashi *et al*., 2007).The liver responds by producing more and pulls cholesterol from the blood after sensing the decrease in bile acids. The

common side effect of this drug is constipation. The potent members of the class include cholestyramine, colestipol and colesevelam (Hou and Goldberg, 2009). Furthermore, cholesterol absorption inhibitors have also been reported for the treatment of hyperlipidaemia which help reduce LDL levels by blocking absorption of cholesterol in the small intestine. Moreover, these drugs have been used in concurrence with a program including eating a low-fat/low-cholesterol diet, exercising and managing weight (Kesaniemi, 2007). However, there is currently no evidence that this drug influences cardiovascular event or mortality rates (Ezetimibe, 2004). However, the class of drugs have been associated with certain side effects like headache, dizziness, stomach upset, joint pain and upper respiratory infections. The potent member of cholesterol absorption inhibitors is ezetimibe. Moreover, Vytorin, a combination medication containing simvastatin and ezetimibe, has been shown to reduce LDL cholesterol to a greater extent than statin therapy alone (Fazio *et al*., 2010). Additional studies are needed to provide more information about the safety and efficacy.Potential treatments for lipid disorders include dietary changes, weight loss, regular exercise, quitting smoking, medications and periodic lipid screenings(Horn, 1997). Bile acid absorption inhibitors, similar to niacin, have been studied mostly in older, pre-statin era trials. In these trials, cholestyramine, a first generation bile acid sequestrant, mainly reduced LDL-C levels as well as cardiovascular events and mortality (Bell and O'Keefe, 2009). However, again like niacin, these drugs have been limited by their gastrointestinal side effects such as constipation (Bell and O'Keefe, 2009). A newer bile acid sequestrant, colesevalam, is tolerated much better by patients and delivers similar LDL-C reductions as the older medications, but no outcome data are available to this point (Corsini *et al*, 2009). Ezetimibe alone has been shown to deliver

modest reductions in LDLC and, in two trials, has been well tolerated by patients intolerant to statins (Gazi *et al*., 2007; Stein *et al*., 2008).

2.3.8.5 Diet Can Play an Important Role in Reducing Serum Cholesterol:

Hereditary factors play the greatest role in determining individual serum cholesterol concentrations; however, dietary and environmental factors also play a part, and the most beneficial of these is the substitution in the diet of polyunsaturated and monounsaturated fatty acids for saturated fatty acids. Plant oils such as corn oil and sunflower seed oil contain a high proportion of polyunsaturated fatty acids, while olive oil contains a high concentration of monounsaturated fatty acids. On the other hand, butterfat, beef fat, and palm oil contain a high proportion of saturated fatty acids. Sucrose and fructose have a greater effect in raising blood lipids, particularly triacylglycerol, than do other carbohydrates (Horn, 1997).

The reason for the cholesterol-lowering effect of polyunsaturated fatty acids is still not fully understood. It is clear, however, that one of the mechanisms involved is the up-regulation of LDL receptors by poly and monounsaturated as compared with saturated fatty acids, causing an increase in the catabolic rate of LDL, the main atherogenic lipoprotein. In addition, saturated fatty acids cause the formation of smaller VLDL particles that contain relatively more cholesterol, and they are utilized by extra hepatic tissues at a slower rate than are larger particles—tendencies that may be regarded as atherogenic (Robert *et al.*, 2006).

2.3.8.6Omega-3 fatty acids

Recently, there has been some interest in the role of omega-3 fatty acids in cardiovascular disease. A large (18000 patients), randomized trial in Japan found that omega-3

supplementation (eicosapentaeonic acid 1800mg/day) significantly reduced triglyceride levels as well as non-fatal coronary events (Yokoyama *et al*., 2010). Adverse effects have included gastrointestinal disturbance, skin reactions, and haemorrhage (Yokoyama *et al*., 2010).

2.3.8.7 Lifestyle Affects the Serum Cholesterol Level:

Additional factors considered to play a part in coronary heart disease include high blood pressure, smoking, male gender, obesity (particularly abdominal obesity), lack of exercise, and drinking soft as opposed to hard water. Factors associated with elevation of plasma FFA followed by increased output of triacylglycerol and cholesterol into the circulation in VLDL include emotional stress and coffee drinking. Premenopausal women appear to be protected against many of these deleterious factors, and this is thought to be related to the beneficial effects of estrogen (Horn, 1997). There is an association between moderate alcohol consumption and a lower incidence of coronary heart disease. This may be due to elevation of HDL concentrations resulting from increased synthesis of apo A-I and changes in activity of cholesteryl ester transfer protein. It has been claimed that red wine is particularly beneficial, perhaps because of its content of antioxidants. Regular exercise lowers plasma LDL but raises HDL. Triacylglycerol concentrations are also reduced, due most likely to increased insulin sensitivity, which enhances expression of lipoprotein lipase. (Robert *et al.*, 2006).

2.3.9 Prevention of hyperlipidemia

2.3.9.1 Primary Prevention: Population-wide Dietary Recommendations

There is little difference between initial dietary recommendations for hyperlipidemia and those for all healthy individuals over the age (Marshall, 1992). There is recognition that

everyone carries some risk of acquiring CHD and its morbidities. The population-wide recommendations promote lifestyle changes along with identifying and modifying risk factors for heart disease. In 2000 the AHA revised its guidelines, which place emphasis on foods rather than nutrients and added weight management as a goal. The AHA guidelines include: ≤30% of calories from total fat intake: <10% of calories from saturated fat intake,<300 mg dietary cholesterol intake/day, two servings of cold water fish per week that are high in omega 3-fatty acids, increase consumption of fruits, vegetables, and low fat and fat-free dairy products (Angela, 2008).

Substitute whole grains, vegetables, fish, legumes and nuts for saturated fat, limit salt to <6 grams per day, limit alcohol ≤2 drinks per day men, ≤1 drink per day women, maintain a healthy body weight, increase physical activity, consume a diet which contains adequate potassium, calcium, and magnesium to facilitate blood pressure control. The 2000 US Dietary Guidelines20(Horn, 1997) also took the focus off of total fat calling the diet "moderate" fat to emphasize saturated fat and cholesterol reduction; they also added a reduction in *trans*-fat.

2.3.9.2 Secondary Prevention: Selective Lipid Screening

A wealth of studies on the population at risk and their dietary habits all point a finger at high saturated-fat diets as being the outstanding coronary-disease-producing factor. Little by little, researchers and public health officials are coming to a revolutionary conclusion(Jones, 1998): Governments must eventually supervise food production—particularly of eggs, dairy products, and meat—to ensure that less saturated fat and cholesterol will be available to the consumer. Most heart research, although intensive, has previously concentrated on the clinical aspects of cardiac disease: how to lower the risk of

heart attack, how to correct heart defects surgically, and how to restore the heart patient to an active life. However, a number of recent discoveries about the biochemistry of the circulatory system may help in the early detection and management of heart disease (Fuller *etal*., 1980).Lipids and lipoproteins are not routinely checked in adolescents and are left to the discretion of the physician. Most screening recommendations suggest measuring lipids in teens that have a strong family history or two or more CHD risk factors.

2.4 Cardiovascular disease

Cardiovascular disease is the class of disease that involves the heart or blood vessels (arteries and veins or both (Maton, 1993). The term technically refers to any disease that affects the cardiovascular system. This disease includes atherosclerosis, myocardial infarction (heart attacks), cerebrovascular diseases, stroke, angina, aneurysm, congestive heart failure, peripheral vascular disease, high blood pressure and coronary artery disease (Bell and O'Keefe, 2009). The causes of cardiovascular diseases are diverse but atherosclerosis and/or hypertension are the most common (Kesaniemi, 2007). Even among healthy asymptomatic individuals, who are aging, come a number of physiological and morphological changes which usually lead to increased risk of cardiovascular diseases (Guyton *et al*., 2008). Cardiovascular diseases and other ailments have been managed by various communities through the administration of medicinal plant products or extracts. Coronary artery diseases (CAD) present some of the major health problems across the globe today, with coronary heart disease, stroke and hypertension being the most common. Death due to coronary heart disease caused by atherosclerosis continues to be a cause of mortality in affluent nations of the world (Johnston *et al*., 2003; Johnston and Waxman, 2008). It is well established that elevated blood lipid levels also known as hyperlipidemia

constitute the primary risk factor for atherosclerosis. For example, elevated levels of cholesterol (hypercholesterolemia), low-density lipoprotein cholesterol (LDL-c) and triglycerides have been implicated. There is now overwhelming evidence that, dietary factor (Horn, 1997), nutritional habits and genetic origin influence the risk of CAD (Kourounakis *etal*., 2002).

Finding sustainable hypolipidemic agents from natural sources that could replace some of the currently available synthetic ones is now receiving much attention. This is crucial owing to a number of side effects associated with the use of synthetic hypolipidemic drugs (Mahmood *etal*., 2010).

2.4.1 Types of cardiovascular disease

Some of the cardiovascular diseases include:

2.4.1.1 Atherosclerosis/ Arteriosclerosis

Arteriosclerosis is disease affecting the walls of the arteries due to ageing (old age) whereas atherosclerosis is a disorder affecting the lumen of the arteries due to plaque build-up. Atherosclerosis is the result of high cholesterol diets and poor exercise while arteriosclerosis is a genetic trait that cannot be changed (Guyton *et al*., 2008).

Atherosclerosis **(**plaque=deposit=thick) is another condition that refers to the deposition of fat plaques and cholesterol globules within the arteries causing narrowing of the lumen of the arteries, In atherosclerosis, there is a gradual increase in the deposition of plaque (consists of cholesterol, lipids, calcium, white blood cells and clumps of platelets) within the lumen causing narrowing or at times complete blockage of the artery. The cause of

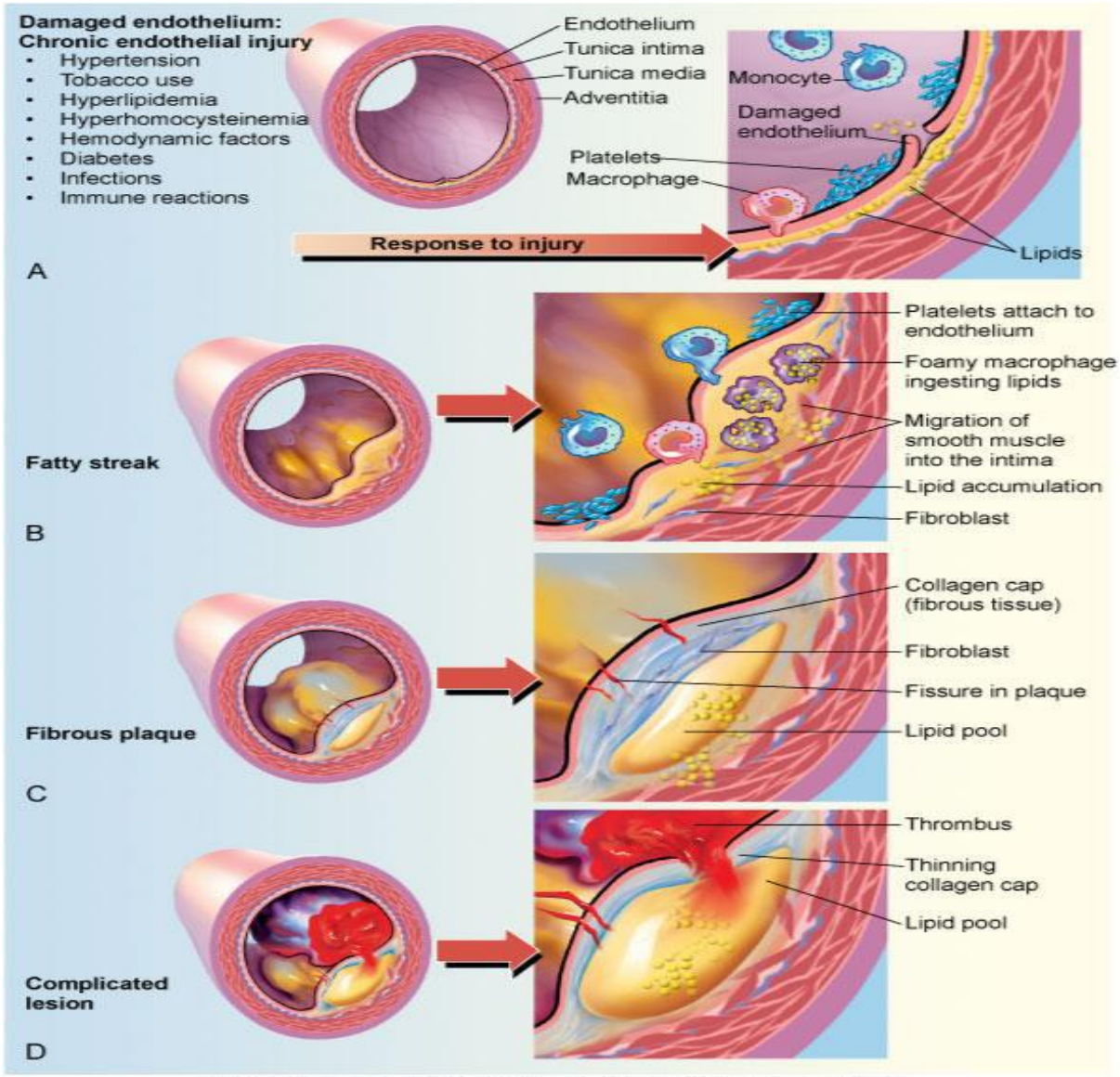
atherosclerosis is not clear but there are multiple predisposing factors like heavy smoking, obesity, diabetes, hypertension, genetic factors, and high serum levels of LDL and cholesterol, high alcohol intake etc.whereas arteriosclerosis **(**hardening=inflexible=inelastic=blood pressure). As age advances, there is loss of protein= elastin causing thickening of the arterial walls. If the walls of the arteries are elastic, they can withstand the turbulence of blood flowing under high pressure which happens when we participate in sports, get angry or overexert in any manner. As the arteries grow harder and thicker, they become more and more resistant to the blood flow and hence, the blood pressure keeps rising as age advances or disease worsens. Arteriosclerosis may or may not narrow the lumen while atherosclerosis always does. There is plaque rupture in atherosclerosis and not in arteriosclerosis.

Atherosclerosis is characterized by the deposition of cholesterol and cholesteryl ester from the plasma lipoproteins into the artery wall. Atherosclerosis remains the major cause of death and premature disability in developed societies. While cholesterol is believed to be chiefly concerned in the relationship, other serum lipids such as triacylglycerol may also play a role. Diseases in which prolonged elevated levels of VLDL, IDL, chylomicron remnants, or LDL occur in the blood (e.g., diabetes mellitus, lipid nephritis, hypothyroidism, and other conditions of hyperlipidemia) are often accompanied by premature or more severe atherosclerosis. Susceptibility to atherosclerosis varies widely among species, and humans are one of the few in which the disease can be induced by diets high in cholesterol (Robert *et al.*, 2006). Current predictions estimate that by the year 2020, cardiovascular diseases notably atherosclerosis, will become the leading global cause of total disease burden. Coronary atherosclerosis causes myocardial infarction and angina

pectoris; strokes and transient cerebral ischemia are due to cerebral atherosclerosis. Intermittent Claudication and gangrene are dreaded sequela of atherosclerosis occurring peripherally.

2.4.1.2 Coronary heart disease

This is due to the narrowing of the blood vessels supplying the heart muscle. These blood vessels are called coronary blood vessels. Atherosclerosis is the most common cause and is caused due to hardening and narrowing of coronary arteries of heart by formation of plaques and plugs. Coronary heart disease can be caused due to risk factors like high blood pressure, high blood cholesterol, tobacco use, obesity, physical inactivity, diabetes and advancing age. Coronary artery disease (CAD) is caused by atherosclerosis of the coronary arteries that leads to a restriction of blood flow to the heart. Atherosclerosis is a process that develops slowly over time. Typically, atherosclerosis begins in a person's teenage years or earlier, and the disease worsens quietly for decades. As people age, their atherosclerosis becomes more likely to involve the arteries of the heart and to become coronary artery disease (WHO, 2015).Obesity is not just a superficial term for excess weight, but has long-standing health implications. As the severity of obesity increase, so too does the risk for coronary heart disease(CHD) –the leading cause of CVD (WHO, 2015).



From Huether SE, McCance KL: *Understanding pathophysiology*, ed 4, St Louis, 2008, Mosby.

Plate 2.1 Chronic damaged endothelial injury.

2.4.1.3 Congenital heart disease

This is due to malformation or abnormal formations of the structures of the heart at birth. This may be inherited or due to other factors. This includes holes in the heart, abnormal valves, heart chambers etc. Mothers taking drugs, alcohol, with infections like rubella or

mothers with poor diets deficient of vital nutrients are at risk of giving birth to babies with congenital heart defects.

2.4.1.4 Stroke or Cerebrovascular accident (CVA)

This is caused when the blood supply to part of the brain is hampered. This may result from either blockage or rupture of a blood vessel in the brain. Postoperative stroke occurs in 1% to 4% of patients. Risk factors include age, previousstroke, diabetes, high blood pressure, high blood cholesterol, tobacco use, diabetes, heart rhythm disorders, hypertension, and female gender (Diodato and Chedrawy, 2014). Along with vital signs, nursingassessment includes postoperative neuro status checks in addition to any functional orcognitive changes that may be due to sudden stroke (Diodato and Chedrawy, 2014).The two major mechanisms causing brain damage in stroke are, ischemia and hemorrhage (Jones*et al.*, 1981).In ischemic stroke, which represents about 80% of all strokes, decreased or absent circulating blood deprives neurons of necessary substrates. The effects of ischemia are fairly rapid because the brain does not store glucose, the chief energy substrate and is incapable of anaerobic metabolism (Jones*et al.*, 1981).Atherosclerosis is the most common pathological feature of vascular obstruction resulting in thrombotic stroke (Challa, 1999).Normal cerebral blood flow (CBF) is approximately 50-to 60 ml/100g/ Min and varies in different parts of the brain (Jones*et al.*, 1981; Pulsinelli, 1995).

2.4.1.5 Congestive heart failure

This is when the heart muscles become progressively unable to pump blood into the blood vessels. Those at risk include those with high blood pressure, heart rhythm disorders, heart attacks and obesity (Challa, 1999).

2.4.1.6 Peripheral arterial disease or peripheral vascular diseases

This affects the arteries supplying the arms and legs. The risks are similar as those for coronary heart disease(World Health Organization, 1994).

2.4.1.7 Deep venous thrombosis (DVT) and pulmonary embolism

In DVT, blood clots form in the leg veins giving rise to severe pain and disability. These clots can dislodge and move to the heart and lungs leading to life threatening complications. Risk factors include long surgery, trauma, obesity, cancers, recent childbirth, use of oral contraceptive and hormone replacement therapy etc. Atherosclerosis is the most common pathological feature of vascular obstruction resulting in thrombotic stroke (Challa, 1999). Atherosclerotic plaques can undergo pathological changes such as ulcerations, thrombosis, calcifications, and intra-plaque hemorrhage countries (Mendis *et al*., 2011). Embolic stroke (ES) can result from embolization of an artery in the central circulation from a variety of sources. Besides clot, fibrin, and pieces of atheromatous plaque, materials known to embolize into the central circulation include fat, air, tumor or metastasis, bacterial clumps, and foreign bodies. Superficial branches of cerebral and cerebellar arteries are the most frequent targets of emboli. Most emboli lodge in the middle cerebral artery distribution because 80% of the blood carried by the large neck arteries flow through the middle cerebral arteries (Garcia, 1998).

2.4.1.8 Rheumatic heart disease

This is caused due to damage to the heart muscle and heart valves from rheumatic fever and infection with streptococcal bacteria.Rheumatic fever(RF) and Rheumatic heart disease (RHD) remain significant causes of cardiovascular diseases in the world today(World

Health Organization, 1994). Rheumatic heart disease is the most common acquired heart disease in children in many countriesof the world, especially in developing countries (Mendis *et al*., 2011). Acute rheumatic fever primarily affects the heart, joints and central nervous system. The major importance of acute rheumatic fever is its ability to cause fibrosis of heart valves, leading to crippling valvular heart disease, heart failure and death (Jones*et al.*, 1981).In 1994, it was estimated that 12 million individuals suffered from RF and RHD worldwide (World Health Organization, 1994), and at least 3 million had congestive heart failure (CHF) that required repeated hospitalisation (Murray and Lopez, 1996). A large proportion of the individuals with CHF required cardiac valve surgery within 5–10 years (World Health Organization, 1994; World Health Organization, 1997).

2.4.1.9 Other cardiovascular diseases

These include tumours of the heart, blood vessel tumours or ballooning (aneurysm) of the blood vessels of the brain, cardiomyopathy, heart valve diseases, disorders of the lining of the heart or pericarditis, aortic aneurysm etc. Chest discomfort or chest pain can originate from many places other than the heart, but thecharacteristic pain of angina almost always points to ischemia of heart muscles (Garcia, 1998).

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

3.1.1 Plant sample collection

The leaves and stem barks of *T. vogelii* were harvested from their natural habitats, Ugboju Agatu, Benue State, Nigeria in July 2012. They were identified/ authenticated at the herbarium unit, Department of Biological Sciences University Agriculture, Makurdi where a voucher specimen number (2234) was deposited.

3.1.2 Equipments, chemicals and reagents

Rotary vacuum evaporator, chromatographic column(octadecylsilane (c-18) of 15 cm length bonded to silica gelfor column 60-230 mesh J. T. Baker) (15-25 micron particle size) and tanks,centrifuge (Donley BS400, England), droppers with rubber suction bulbs, shaker (Stuart Scientific Orbital Shaker, Essex, UK), Silica gel 60 F_{254} plates (Merck) TLC (20cm x 20cm) , GC-MS (model GC-MS-QP-2010 plus, shimadzu make), FTIR-8400S,0.03mm sieve (BS 410 Endecotts Ltd London),atorvastatin (Pfizer lreland Pharmaceuticals, cages (47x34 x 18 cm), lreland),One pipette delivering 100-1000µl,Disposable tips (100µl, 500µl) for measuring aliquots of dilute solutions, measuring cylinder,poloxamer 407 (N.F. (Pluronic F-127; Sigma-Aldrich Company 3050 Spruce Street, M.O, U.S.A.).

All assay kits were from Randox laboratory Ltd. Ardmore, Co. Antrum UK. Chemicals and reagents to be used were purchased from (Sigma chemical company St. Louis U.S.A.) and were of analytical grade, according to the manufacturers' instruction.

3.1.3 Experimental animals

Permission was granted by animals' caretakers of Department of Pharmacology, Vom, and Jos Plateau state to obtain some rats for scientific research. A total of one hundred and forty eight (148) male wistar albino rats (*Rattus noruegicus*) of approximately twomonths old weighing between 90-130g were bought from National Institute of Tryapanosomiasis Research (NTIR), Vom, Jos Plateau state.

3.2 Methods

3.2.1: Preparation of plant sample

The fresh leaves and stem bark were sorted out and washed to remove particles and dust. The washed parts were dried using atmospheric air. The dried parts were ground into powder using mortar and pestle before being separately milled into fine powder using an electric blender to pass through a sieve. The sample was sieved with 0.03mm sieve made of brass material and the powder obtained was then used for subsequent analyses.

3.2.2 Animals

The animals were housed inawellaerated clean large plastic cages lined with husk renewed every 24 h, at a room temperature of 25^0C, relative humidity of 75 and 12hrs dark-light cycle.The animals were fed with grower and starter mash purchased from vital feeds company in Zaria, Kaduna State, Nigeria. Composition of the experimental diet (54% carbohydrate, 10% protein 10% fats, 20% fibre, 4% minerals and1% vitamin) and water was provided *adlibitum* throughout the experiment and allowed to acclimatize for 2 weeks before beginning the experiment. Doses were chosen after carrying out toxicity experiments as described by Lorke (1983) using doses ranged between 10 and 5000 mg/kg.

3.2.3 Qualitativephytochemical screening of plant materials

Chemical tests were carried out on the powdered sample using standard procedures to identify the constituents that are active as described by Sofowara (1993),Evans and Trease (2002) and De *et al.*, (2010).

i) Test for tannins:

Dried sample(0.5 g) of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. Three (3) drops of 0.1% ferric chloride, $FeCl_3$ was added and observed for brownish green or a blue-black colouration which showed the presence of tannins.

ii) Test for saponins:

Powdered sample (2g) of plant was boiled together with 20ml of distilled water in a water bathand filtered. About 10ml of the filtered sample was mixed with 5ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing is then mixed with 3 drops of olive oil and for the formation of emulsion which indicates the presence of saponins.

iii) Test for phlobatannins:

Powdered sample (2g) of the plant was boiled with 1% HCl acid in a test tube or conical flask. If the sample of plant contained phlobatannins, a deposition of a red precipitate occurs which indicates the presence of phlobatannins.

iV) Test for alkaloids

Powered sample(1.0g) was stirred with 5ml of 1% aqueous HCl on a steam bath and filtered. Then 1ml of the filtrate was treated with a few drops of Mayer's reagent and

another 1ml portion similarly treated with Wagner's reagent. The cream or pale yellow precipitate was observed as evidences of presence of alkaloids.

v) Test for steriods:

Two ml of acetic anhydride was added to 0.5 g powdered sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

vi)Test for terpenoids (Salkowski test):

Five grams (5g) of powdered sample was mixed in 2 ml of chloroform, $CHCl_3$ in a test tube and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

vii) Test for flavonoids:

Powered plant (0.5g)sample, was heated with 10ml of ethyl acetate over steam bath for 3min. The mixture was shaken with 1ml of dilute ammonia solution. The yellow colouration was observed as evidence of flavonoids.

viii) Test for anthraquinones

Powdered sample(1.0g) was shaken with 5ml benzene, filtered and 1ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the brown layer formed showed the presence of free anthraquinones (Borntragers Test).

3.2.4 Preparation of extracts

Based on the sample to solvent ratio of 1:10 (w/v) (Das *et al*., 2010), 100g of each of the ground samples (leaves and stem bark) were suspended in 1000ml of different extraction solvents in 100% distilled water, acetic acid and 70% ethanol on a shaker for 48 hrs at room

temperature. Each extract was filtered using a sterilized Buchner funnel and Whatman No. 1 filter papers. The filtrates were concentrated by drying in a water bath maintained at a temperature of 45^0C until a brownish black residue were obtained and the weights of the extracts were determined asthe percentage weight of the extract to the original weight of the sample used, using the formular below.The extracts were kept in the sealed containers and refrigerated at 2-4^0C from where aliquots were reconstituted for the experiment.

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of original sample}} \times \frac{100}{1}$$

3.2.5*In vivo* biological screening of the extracts

Six different solvent extracts (aqueous, acetic acid and 70% ethanol) of leaves and stem were used for in vivo study to ascertain the extract with the highest antihyperlipidaemic activity.

3.2.5.1: Animal grouping

A total of forty(40) apparently healthy rats were used for the study.The rats were shared into 10 groups of 4 rats each and the induction was lasted for three weeks after they were confirmed to be hyperlipidemic. Extracts of the same concentration (1g/25ml) were prepared and 50mg/kg body weight was administered to the rats. Treatment with the extractsorally lasted for 20 days. On the 21st day, the animals were sacrificed and blood sample was collected for lipid profile.

Group1: Normal rats administered distilled water and feed only (normal control).

Group2: induced with Poloxamer 407 (0.5g/kg b.wt) + distilled water and feed only (Hyperlipidemic control).

Group3: induced with High Fat Diet + distilled water and feed only (hyperlipidemic control).

Group4: Hyperlipidemic rats, administered 10 mg/kg body weight of Atorvastatin (Kumar *et al*., 2010).

Group5: Hyperlipidemic rats, administered 50mg/kg body weight of aqueous leaf extract

Group6: Hyperlipidemic rats, administered 50mg/kg body weight of aqueous stem extract

Group7: Hyperlipidemic rats, administered 50mg/kg body weight ofethanolic leaf extract

Group8: Hyperlipidemic rats, administered 50mg/kg body weight ofethanolic stem extract

Group9: Hyperlipidemic rats, administered 50mg/kg body weight ofacetic acid leaf extract

Group10: Hyperlipidemic rats, administered 50mg/kg body weight ofacetic acid stem extract

3.2.6 Induction of hyperlipidemia

3.2.6.1 Induction using poloxamer 407

Hyperlipidemia was induced with the method described by Megalli *etal*., (2005). Healthy wistar albino rats (Rattus norvegicus) of approximately two months old males weighing between 80- 130g were randomly assigned to ten groups (4 rats per group). Poloxamer 407, solution for intraperitoneal (i.p.) injection was prepared by combining the agent with distilled water. This was refrigerated overnight to facilitate dissolution of the P407 by the cold method of incorporation (Schmolka, 1991). Administration P407 (0.5 g/kg) was by I.p. injection for every two days for 21 days. The rats were weighed three times per week and food intake in grams was monitored.

All syringes were placed on ice prior to Poloxamer 407 administration to maintain the polymer in a mobile viscous state during the injection. To confirm the induction of hyperlipidemia, blood samples were collected via retro orbital puncture of the induced animals after 2 hrs of administration; total cholesterol and triacylglycerol concentrations of the blood samples were determined using a standard diagnostic kit. Rats total cholesterol and triglyceride levels above 200mg/dl(6.2mmol/L) and 160mg/dl(1.7mmol/L) respectively were considered hyperlipidemia (NCEP, 2001; Delvin, 2006; Suzuki, 2006).

3.2.6.2 Induction using high fats diet

The standard diet was prepared by blending the standard animal diet. High fat diet cocktail was prepared by mixing cholesterol (100g), cholic acid (50g) in 1 liter of coconut oil supplemented with egg (Jain *etal*., 2008). The animals were given this high fat died cocktailorally for 21 days alongside normal chow.

3.2.7 Preparation of standard drug

Atorvastatin was purchased in a tablet form at a strength of 10mg. Tablets were dissolved in 20ml of normal saline to desired concentrations and administered orally. This preparation was done daily at time of administration.

3.2.8Termination, collection and preparation of sera samples

At the end of the 21 days animals were fasted for 12 hours, the animals were sacrificed by chloroform-inhalation anesthesia (Shah*etal*., 2010) and the blood sample was collected from the heart of each into plain bottles (for biochemical analysis) and vacutainers containing Ethylene diamine tetraacetic acid (EDTA). The blood samples collected in plain sample bottles were centrifuged at a speed of 300 x g for 10 minutes and the supernatant (serum) collected was then subjected to biochemical analysis.

3.2.9Determination of biochemical parameters

3.2.9.1 Serum total cholesterol (TC) concentration

The value of serum level of total cholesterol was quantified by spectrophotometric methods as described by Steinberg and Witztum (2011) by the addition of enzyme present in reagent kit.

Assay Principle: A large portion of the cholesterol in blood is in the form of cholesteryl esters, which is hydrolysed by cholesterol esterase into cholesterol and fatty acids. Cholesterol is then oxidized by cholesterol oxidase to yield H_2O_2. The H_2O_2 produced interact with a sensitive cholesterol probe to produce Quinoneimine, which can be detected spectrophotometrically. The value of TC present in serum will be expressed in the unit of mg/dl.

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator Quinoneimine is formed from Hydrogen Peroxide and 4-amino antipyrine in the presence of phenol and peroxidase.

Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ Cholestene-3-one + H_2O_2

$2H_2O_2$ + Phenol + 4-Amino antipyrine $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_2O$

Procedure: Distilled water (10µl), cholesterol standard (10µl) and test serum (10µl) were dispensed into the test tubes labelled reagent blank, standard and sample respectively. One millilitre of the cholesterol reagent was then added to each of the tubes and mixed. The mixture was incubated at 37^{o}C for 5 minutes in a water bath. The absorbance of the sample (A_{sample}) and that of the standard ($A_{standard}$) were measured against the reagent blank at

500nm wavelength using a colorimeter. The values obtained were used to calculate the total cholesterol concentration using the formula:

Total cholesterol (mg/dl) = $\frac{A_{sample}}{A_{standard}}$ X Concentration of standard

3.2.9.2 Serum triacylglycerol (TG) concentration

The serum triacylglycerols level was also determined by method of Heber *et al*.,(2013). Using Randox kit (Randox Laboratories Limited UK). In these methods triglycerides is converted into quinoneimine dye. Quinoneimine dye is formed at a rate proportional to triglyceride concentration in the serum. Since it is a coloured compound it can be detected and quantified by a colorimeter. The value will be expressed in the unit of mg/dl.

Assay Principle: The triacylglycerol was determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-amino phenazone and 4-chlorophenol under the catalytic influence of peroxidase.

Triacylglycerol + H_2O $\xrightarrow{\text{Lipase}}$ glycerol + Fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 $\xrightarrow{\text{Glycerophosphate Oxidase}}$ dehydroxyacetone phosphate + $2H_2O_2$

$2H_2O_2$ + 4-Aminophenazone + 4-Chlorophenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_2O$ + HCl

Procedure: One millitre of the Randox TG enzyme reagent (containing a mixture of lipase, glycerol-kinase, ATP, 4-aminophenazone, 4-chlorophenol, peroxidase, and glycerol-3-phosphate oxidase and magnesium ions) was dispensed into three separately labelled test tubes; standard, sample and reagent blank. The standard, contained 10µl test serum while that labelled reagent blank contained 10µl of distilled water. The mixture was incubated for

5 minutes at 37^{o}C in a water bath. After the period of incubation the absorbance of the sample (A_{sample}) and that of the standard ($A_{standard}$) was then read against the reagent blank within 60 minutes using a colorimeter at 500nm wavelength. The triacylglycerol concentration was calculated using the formula:

Triacylglycerol concentration (mg/dl) = $\frac{A_{sample}}{A_{standard}}$ X Concentration of standard

3.2.9.3 Serum high density lipoprotein-cholesterol (HDL-C) concentration

The serum level of HDL-C was measured by the method described by Wacnic and Albers (1978). The value was also be expressed in the unit of mg/dl.

Assay Principle

Low density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample was precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 4000 rpm after centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined.

Calculation/HDL-c concentration = concentration of standard

3.2.9.4 Serum low density lipoprotein-cholesterol (LDL-C) concentration

The serum level of LDL-C was measured according to protocol of Friedewald *etal*., (1972) using this equation: LDL-c (mg/dl) =TG/5-HDL-c. The value was expressed in the unit of mg/dl.

1) LDL mg/dl = Total cholesterol – HDL - TG/5.

2) Atherogenic Index (AI) $= \frac{\text{Total cholesterol} - \text{HDL}}{\text{HDL}}$

3.2.10Quantitative determination of phytochemical constituents in the most potent extract (aqueous leaves) of *T. vogelii*

i) Saponins determination

Saponins in the extract was determined using the method of Obdoni and Ochuko,(2001). The aqueous extract (15 g) was added to 100 ml of 20% aqueous ethanol and kept in a shaker for 30 min. The sample was heated over a hot water bath for 4 h with continuous stirring at 55^{o}C. The mixture was then filtered and the residue re-extracted with another 200 ml of 20% aqueous ethanol. The combined extract was reduced to approximately 40 ml over the water bath at 90^{o}C. The concentrate was transferred into a 250 ml separatory funnel and extracted twice with 20 ml diethyl ether. The ether layer was discarded while the aqueous layer was retained and to which 60 ml n-butanol was added. The n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation, the sample was dried in the oven at 40^{0}C to a constant weight. The saponins content was calculated using the formula: % saponins = final weight of sample/initial weight of extracts x 100.

ii)Determination of alkaloid

Alkaloid was determined according to the method of Harborne (2005): Five (5 g) of the aqueous extract was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the filtrate was concentrated on a water bath to one-fourth of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was completed. The

whole solution was allowed to settle and the precipitates were collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed. The alkaloid content was determined using the formula: % alkaloid = final weight of the sample / initial weight of the extract x 100.

iii) Determination of total phenols

Totalphenols determination was done by the method of Boham and Kocipai- Abyazan (1994): 15 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight. The total phenol content was determined using the formula: % total phenol = final weight of the sample / initial weight of the extract x 100.

iv)Determination of flavonoid

Flavonoid determination was done by the method of Boham and Kocipai- Abyazan (1994): 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight. The flavonoid content was determined using the formula: % flavonoid = final weight of the sample / initial weight of the extract x 100.

3.2.11 Lethality (LD_{50}) test of aqueous leaves extract

The mean lethal dose (LD_{50}) of the aqueous leaves extract was conducted in order to select a suitable dose for the evaluation of antihyperlipidemic activity. This was done using the method described by Lorke (1983) using 13 rats.

In the first phase, 9 rats were divided into 3 groups of 3 rats each and were treated with 10mg/kg, 100mg/kg and 1000mg/kg of the extract orally. They were observed for 24hr for signs of toxicity (response or effect) including death.In the second phase, 4 rats were divided into 4 groups of one rat each, and were given doses of the extract per kg body weight based on the findings in phase 1. The doses given in the second phase were 1000mg/kg, 1600mg/kg, 2900mg/kg and 5000mg/kg. From the result of phase 1 and the phase 2, the $LD_{50}$was calculated as the square root of the geometric mean of the highest non-lethal dose and the lowest lethal dose.

LD50 = $\sqrt{Highest\ non-lethal\ dose\ X\ lowest\ lethal\ dose}$

3.2.12Seperation of the most potent extract

Column chromatography

Seperation of the active component(s) of the aqueous leaves extract with highest activity was carried out using a reversed phase chromatography column.Crude extract was fractionated bymeans of column chromatography using silica gel 60-120 mesh size.Twenty five gm silica gel was used for making slurry with petroleumether (60-80^{o}C) and loaded in a glass column of length 30cm andinternal diameter 2cm. Each time 0.5gm of crude sample was loaded. The column was conditioned by washing with 10ml of redistilled methanol followed by the 10ml distilled water at a flow rate of 0.5mL/min. This process was to remove any impurity that was trapped within the column. The redistilled methanol was

applied to the column in aliquots and allowed to run through. A portion of dried crude aqueous extract, 5.0g was emptied into a clean porcelain mortar and 5ml of PBS was added. Pestle was then used to macerate the mixture to homogeneity. The mixture was carefully transferred into a labelled sample bottle to the last drop. Another 5ml of PBS was added to the residue in the mortar and macerated again for further extraction. The resultant mixture was then added to the previous labelled sample bottle to the last drop and filtered using filter paper or centrifuged to remove particulates that could potentially clog the column. The filtrate was then chromatographed by applying the extract to column in aliquots using the Pasteur pipette.

The column was loaded with the 4ml of filtrate in aliquots using Pasteur pipette. After loading, effluent was collected and 10ml of distilled water was run through the column and collected as washings. The column was then eluted with 4ml of the best solvent system (Graded) solution of % (v/v) of methanol in water, 10, 20, 30,40,50,60,70,80,90 and 100% (v/v). Elution of the compounds involved the application of 4ml each of the solvent mixture into the column using a calibrated of 5ml syringe. The resultant eluates (fractions) were also collected in 4ml fraction with the aid of a graduated measuring cylinder. The collected 4ml eluates were evaporated to near dryness in a rotary evaporator under vacuum, and dried further on a lyophilizer .The eluates and effluent were kept in the refrigerator for further analysis. When the elution process was completed the column was regenerated. Regeneration of the column involved washing the column with 20ml of acetone followed by 20ml of redistilled methanol (Kitagawa, 1986; Price *et al*., 1987; Curl *et al*., 1988; Oleszek, 1988).The collected 4ml eluates were evaporated to near dryness in a rotary

evaporator under vacuum, and dried further on a lyophilizer and the weights of the eluates were determined asthe percentage weight of the eluates to the original weight of the sample used, using the formular below.

$$\text{Percentage yield} = \frac{\text{Weight of Eluate}}{\text{Weight of original sample}} \times \frac{100}{1}$$

3.2.13Screening of the ten fractions from column chromatographyfor anti-hyperlipidemia

A study was conducted using ten fractions (F1-F10) obtained from the methanol in water leaf extract of *T. vogelii*to screen for antihyperlipidemic activity in poloxamer-induced hyperlipidemic rats.

3.2.13.1: Animal grouping for screening of ten fractions

A total of fifty two (52) apparently healthy rats were used for the study. The rats were divided into 13 groups of 4 rats each and the induction was lasted for three weeks after they were confirmed to be hyperlipidemic as described in section 3.2.6.1. Atorvastatin was administered orally as described in section 3.2.7.Ten fractions of the same concentration (0.16g/10ml) were prepared and 50mg/kg body weight was administered to the rats.At the end of the 21 days treatment was terminated, the animals were sacrificed and the sample was collected as described in section 3.2.8. The supernatant (serum) collected was then subjected to biochemical analysis as described in section 3.2.9.

Group1: Normal rats administered distilled water and feed only (normal control).

Group2: induced with Poloxamer 407 (0.5g/kg b.wt) + distilled water and feed only (Hyperlipidemic control).

Group3: Hyperlipidemic rats, administered 50mg/kg body weight of fraction 1(F1)

Group4: Hyperlipidemic rats, administered 50mg/kg body weight of fraction 2(F2)

Group5: Hyperlipidemic rats, administered 50mg/kg body weight of fraction 3(F3)

Group6: Hyperlipidemic rats, administered 50mg/kg body weight of fraction 4(F4)

Group7: Hyperlipidemic rats, administered 50mg/kg body weight of fraction 5(F5)

Group8: Hyperlipidemic rats, administered 50mg/kg body weight of fraction 6(F6)

Group9: Hyperlipidemic rats, administered 50mg/kg body weight of fraction 7(F7)

Group10: Hyperlipidemic rats, administered 50mg/kg body weight of fraction 8(F8)

Group11: Hyperlipidemic rats, administered 50mg/kg body weight of fraction 9(F9)

Group12: Hyperlipidemic rats, administered 50mg/kg body weight of fraction 10(F10)

Group13: Hyperlipidemic rats, administered 10 mg/kg body weight of Atorvastatin.

The fraction with most potent antihyperlipidemic activity was used for further study

3.2.14 Phytochemical screening of fraction F6

Standard protocols (Sofowora, 1993; Evans and Trease 2002) were used in detecting the presence of different phytochemical constituents in fraction F6 as described in section 3.2.3.

3.2.15: Antihyperlipidemic effect of fraction 6 and aqueous leaves extract

3.2.15.1 Animal grouping and treatments

The rats were shared into 7 groups of 4 rats each and the induction of twenty four (24) apparently healthy rats was lasted for three weeks after they were confirmed to be hyperlipidemic as described in section 3.2.6.1. Atorvastatin was administered orally as described in section 3.2.7. The treatment was orally using the feeding tube for 21 days.

Group1: Normal rats administered distilled water and feed only (normal control).

Group2: Hyperlipidemic rats, administered distilled water and feed only (hyperlipidemic

control).

Group3: Hyperlipidemic rats, administered 10 mg/kg body weight of Atorvastatin.

Group4: Hyperlipidemic rats, administered 100mg/kg body weight of aqueous leaf extract

Group5: Hyperlipidemic rats, administered 200mg/kg body weight of aqueous leaf extract

Group6: Hyperlipidemic rats, administered 100mg/kg body weight of fraction 6(F6)

Group7: Hyperlipidemic rats, administered 200mg/kg body weight of fraction 6(F6)

3.2.15.2Termination, collection and preparation of sera samples and organs extract

At the end of the 21 days, animals were fasted for 12 hours and sacrificed by chloroform-inhalation anesthesia.The blood sample was collected from the heart of each into plain bottles (for biochemical analysis). The blood samples collected in plain sample bottles were centrifuged at a speed of 300 x g for 10 minutes and the supernatant (serum) collected was then subjected to biochemical analysis. Immediately after collection of blood, Organs (liver and kidney) were excised and kept in the PBS for further analysis in the refridgerator.

3.2.16Determination of biochemical parameters

3.2.16.1 Lipid profile

Lipid profile (TC, TAG, HDL-c and LDL-c) was determined as described in sections 3. 2. 9. 1- 3. 2. 9. 4.

3.2.16.2 Serum aspartate aminotransferase (AST) activity

The serum aspartate aminotransferase activity was determined as described by Reitman-Frankel end- point technique (1957), using assay kits (Randox laboratories Ltd) and expressed in U/l.

Assay principle: Aspartate aminotransferase was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4 dinitrophenylhydrazine at 540 nm and 37^0C.

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

Oxaloacetate + 2, 4-dinitrophenylhydrazine $\longrightarrow$ Oxaloacetate hydrazone

*Procedure:*Exactly, 0.5ml of reagent 1 which was made up of phosphate buffer, L-aspartate and α-oxoglutarate was added into a clean test tubes: one containing 0.1m1 of distilled water (Reagent blank) and the other 0.1ml of test serum (sample). The mixture was incubated for exactly 30 minutes at 37^0C in a water bath. About 0.5m1 of reagent 2 which was made up of 2,4-dinitropheny1hydrazine was added to each of the test tubes and mixture was allowed to stand for exactly 20 minutes at 20-25^0C in a water bath. At the end of 20 minutes, 5m1of sodium hydroxide solution (0.4mol/l) was added to each of the test tubes, mixed and absorbance of the sample (A_{sample}) was read against the blank (also prepared) using a colorimeter at 546 nm after 5 minutes. The activity of AST concentration (u/l) in the serum was determined by extrapolating the corresponding absorbance from the plot of the standard calibration curve for the enzyme provided in the manual of Randox lab. Ltd, UK Reagent kit.

3.2.16.3 Serum alanine aminotransferase (ALT) activity

The serum alanine aminotransferase activity was determined as described by Reitman - Frankel end -point technique (1957), using assay kits (Randox laboratories ltd) and expressed in U/l.

*Assay principle:*Alanine aminotransferase was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4- dinitrophenylhydrazine at 540 nm and 37^0C.

α-ketoglutarate + L-alanine $\xrightarrow{\text{ALT}}$ L-glutamate + pyruvate

Pyruvate + 2, 4- dinitrophenylhydrazine $\longrightarrow$ pyruvate hydrazone

*Procedure:*Exactly, 0.5ml of reagent 1 which was made up of phosphate buffer, L-alanine and α-oxoglutarate was added into clean test tubes: one containing 0.1m1 of distilled water (Reagent blank) and the other 0.1ml of test serum (sample). The mixture was incubated for exactly 30 minutes at 37^0C in a water bath. 0.5m1 of reagent 2 which was made up of 2, 4-dinitropheny1hydrazine was added to each of the test tubes and mixture was allowed to stand for exactly 20 minutes at 20-25^0C in a water bath. At the end of 20 minutes, 5m1of sodium hydroxide solution (0.4mol/l) was added to each of the test tubes, mixed and absorbance of the sample (A_{sample}) was read against the blank (also prepared) using a colorimeter at 546 nm after 5 minutes. The activity of ALT concentration (u/l) in the serum was determined by extrapolating the corresponding absorbance from the plot of the standard calibration curve for the enzyme provided in the manual of Randox lab. Ltd, UK Reagent kit.

3.2.16.4 Serum alkaline phosphatase (ALP) activity

The serum level of alkaline phosphatase activty was quantified by optimized standard method described by Haussament (1977) using Randox lab. Ltd, UK Reagent kit.

Assay principle

The alkaline phosphatase activity was measured by monitoring the concentration of phosphate hydrazone formed with 2, 4- dinitrophenylhydrazine at 405 nm 37^0C.

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

Phosphate + 2, 4-dinitrophenylhydrazine $\longrightarrow$ Phosphate hydrazone

*Procedure:*Exactly, 0.5ml of reagent 1 which was made up of phosphate buffer, alkaline and α-oxoglutarate was added into a clean test tubes: one containing 0.1m1 of distilled water (Reagent blank) and the other 0.1ml of test serum (sample). The mixture was incubated for exactly 30 minutes at 37^0C in a water bath. 0.5m1 of reagent 2 which was made up of 2,4-dinitropheny1hydrazine was added to each of the test tubes and mixture was allowed to stand for exactly 20 minutes at 20-25^0C in a water bath. At the end of 20 minutes, 5m1of sodium hydroxide solution (0.4mol/l) was added to each of the test tubes, mixed and absorbance of the sample (A_{sample}) was read against the blank (also prepared) using a colorimeter at 546 nm after 5 minutes. The activity of ALP concentration (u/l) in the serum was determined by extrapolating the corresponding absorbance from the plot of the standard calibration curve for the enzyme provided in the manual of Randox lab. Ltd, UK Reagent kit.

3.2.16.5Serum total and direct bilirubin concentration

Total bilirubin (TB) and direct bilirubin (DB) was estimated by acid diazo method as described by Doumas *etal*., (1973), using assay kits (Radox Laboratories Ltd).

*Assay Principle:*Sulphanilic acid was diazotized by the nitrous acid produced from the reaction between sodium nitrite and hydrochloric acid. Direct (conjugate) bilirubin reacts with diazotized sulphanilic acid (diazo reagent) in alkaline medium to form a blue colour complex (azobilirubin). The pink acid azobilirubin is converted to blue azobilirubin by an alkaline tartrate. Total bilirubin was determined in the presence of caffeine, which releases albumin bound bilirubin by the reaction with diazotized sulphanilic acid. Indirect bilirubin, Diazotized sulphanilic acid, later measured calorimetrically.

*Procedure:*For total bilirubin; exactly, 0.5ml of reagent 2 was made up of sodium nitrate and added into a clean test tube containing 0.2ml of reagent 1 which was made up of sulphanilic acid and hydrochloric acid and 1.0ml of reagent 3 made up of caffeine and sodium benzoate was added followed by 0.2ml of serum, mixed and allowed to stand for 10 minutes at room temperature. About 1ml of reagent 4 made up of tartrate, and sodium hydroxide was also added, mixed and allowed to stand for 10 minutes at room temperature. The absorbance of the sample against sample blank (ATB) was read at 560 nm. For direct bilirubin; 0.5ml of reagent 2 was added into a clean test tube containing 0.2ml of reagent 1, then 2.0ml of sodium chloride (9g/l) was added followed by 0.2ml of serum, mixed and allowed to stand for 5 minutes at room temperature. The absorbance was read against the sample blank (ADB) at 530nm.

Calculation

Total bilirubin (mg/dl) =10.8 x ATB

Direct bilirubin (mg/dl) =14.4 x ADB

Unconjugated bilirubin (mg/dl) =10.8 x ATB- 14.4 x ADB

ATB = absorbance of total bilirubin, ADB = absorbance of direct bilirubin

3.2.16.6Serum creatinine concentration

Colourimetric method was used to determine serum creatinine concentration according to Bartels and Bohmer (1973) using Randox assay kits.

Principle: Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is proportional to the creatinine concentration.

Reagent Composition: CAL. Standard (2.06 mg/dl), Rla: Picric Acid (35 mmol/l), Rlb: Sodium Hydroxide (0.32 mol/l).

Reagent Preparation: An equal volume of solutions Rla + Rlb was mixed.

Procedure: Exactly1ml of working reagent containing picric acid and sodium hydroxide was added into two clean test tubes labelled sample test and standard, containing 0.1ml of sample and 0.1ml of standard solution. The content in each test tube was mixed and after 30 seconds, the absorbance A_1 of the standard and sample was read. Exactly 2 minutes later, absorbance A_2 of the standard and sample was read at 490nm.

Calculation

The Concentration of creatinine in serum was calculated using the formular below;

$\frac{\Delta \mathbf{A} \text{ sample}}{\Delta \mathbf{A} \text{ standard}} \times \text{Standard conc. (mg/dl)} = \text{mg/dl}$

$A_2 - A_1 = \Delta\mathbf{A}$ sample or $\Delta\mathbf{A}$ standard

3.2.16.7Serum urea concentration

Principle: The serum urea concentration was determined using urea Randox assay kit. Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia formed is then measured photometrically by Berthelot‟s reaction (Fawcett and Scout, 1960).

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

NH_3 + hypochlorite + phenol indophenols (blue compound).

Reagent Composition: R1: EDTA (116mmol/l), Sodium nitroprusside (6mmol/l), Urease (1mmol/l). R2: Phenol (diluted) (120mmol/l). R3: Sodium hypochlorite (diluted) (27mmol/l), NaOH (0.14 N). CAL. Standard: (80.65 mg/dl).

Reagent Preparation: R1. Sodium nitroprusside and urease (Solution Rl). The contents of vial Rla was transferred into bottle Rlb and mixed gently. R2 (Phenol) content of bottle R2 was diluted with 660 ml of distilled water. R3 (Sodium hypochlorite) content of bottle R3 was diluted with 750 ml of distilled water.

Procedure: Exactly 100 µl of reagent 1 containing sodium nitropusside and urease was added into three clean test tubes labelled as test sample, standard and reagent blank containing 10 µl sample, 10 µl standard reagent and 10 µl distilled water respectively. The content in each of the test tube was mixed and incubated at 37^{o}C for 10 minutes, 2.5ml of the reagent 2 containing diluted phenol and reagent 3 containing diluted sodium hypochlorite and sodium hydroxide were added to each of the three test tubes, the content in each of the test tube was mixed immediately and incubated for 15 minutes. The absorbance of the test sample (A sample) and standard (A standard) were read against blank at 500nm.

Calculation

The serum urea concentration was calculated using the formular below;

$$\text{Urea Concentration} = \frac{\Delta \mathbf{A} \text{ sample}}{\Delta \mathbf{A} \text{ standard}} \times \text{Standard Conc. (mg/dl)}$$

$\Delta \mathbf{A}$ sample = Change in absorbance for sample

$\Delta \mathbf{A}$ standard = Change in absorbance for standard

3.2.17 Determination of*in vivo*antioxidant property

Immediately after the blood was collected, liver and the kidney were quickly and carefully dissected out and the blood was wiped with tissue. One gramme (1g) of the liver and

kidney was weighed and crushed using mortar and pestle. Ten millilitre 10ml Phosphate buffered saline (PH 7.4) was added to obtain 10% (w/v) homogenates. The resulting homogenates were centrifuged at 4000g for 10 minutes. Then the supernatant was collected using Pasteur pipette for the measurement of scavenging enzyme activities; malondialdehyde (MDA) (Fraga *et al*., 1988), superoxide dismutase(SOD) (Martin *et al*., 1987), catalase (Mahmoud, 2016) and reduced glutathione (GSH) (Ellman, 1959).

3.2.17.1 Superoxide dismutase (SOD) activity

Superoxide dismutase activity of extract was measured using the method described by Martin *etal*., 1987. Auto-oxidation of heamatoxylin (with increase in absorbance at 560 nm) is inhibited by SOD activity at the assay pH 7.8; the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range. SOD activity in the sample was determined by measuring the amount of heamatin at 560 nm.

Heamatoxylin $\longrightarrow$ H_20+ Heamatin (active form of heamatoxylin)

To 920ul of phosphate buffer (0.05M, pH 7.8) was added 40ul of sample. A reagent test was also prepared by replacing the sample with 40ul of sample dilution buffer (0.85% NaCl). The mixtures were incubated for 2min at 25^0C before the addition of 40ul of hematoxylin. Following the addition of 40ul hematoxylin, absorbance of the sample test and reagent test were read at 560 nm immediately and after 5 minutes against the blank which was distilled water.

SOD concentration in the sample was calculated thus:

Absorbance $_{\text{Reagent test}}$ (A_R) = Absorbance $_{\text{Reagent test2}}$- Absorbance $_{\text{Reagent test1}}$

Absorbance $_{\text{Sample test}}$ (A_S) = Absorbance $_{\text{Sample test 2}}$ - Absorbance $_{\text{Sample test 1}}$

% inhibition= $[1-A_S/A] \times 100$

SOD (u/ml) = [1-A_S/A_R] x 100 x1.25

3.2.17.2 Catalase activity

Catalase (CAT) activity was determined using the method described by Mahmoud, (2016).

*Principle:*Catalase scavenges hydrogen peroxide converting it to water and molecular oxygen. The concentration of H_2O_2 in the absorbance reading after 10min was determined as catalase activity expressed in terms of units/mg protein.

$2H_2O_2 \longrightarrow 2H_2O + O_2$

The activity of catalase in the sample was determined by following the rate of decrease in absorbance at 240 nm.

*Procedure:*Tissue homogenate of about 10ul (100-150ug protein) was added to 2.8 ml of 50Mm potassium phosphate buffer (pH 7.0) in 3 ml cuvette. The reaction was initiated by adding 0.1ml of fresh 30 Mm H_2O_2 and the decomposition rate of H_2O_2 was measured at 240 nm for 5 minutes on a spectrophotometer. A molar extinction coefficient of 0.041 $mM^{-1}cm^{-1}$ was used to calculate catalase activity which was expressed as mole H_2O_2 decreased/min/mg protein.

3.2.17.3 Estimation of reduced gluthathione (GSH) and gluthathione peroxidase (GSPX) Activity

Reduced glutathione (GSH) was determined by the method of Ellman, (1959). One milliliter of supernatant was taken after precipitating 0.5ml of sample with 2ml of 5% TCA. To this, 0.5ml of Ellmans reagent (0.0198% DTNB in1% sodium citrate) was added and read at 412nm. Reduced GSH concentration was measured by using a drawn standard curve and expressed as mg/g of tissue.

3.2.17.4 Thiobarbituric acid reactive substances (TBARS)concentration

Thiobarbituric acid reactive substances (TBARS) in the tissues were estimated using the method of Fraga *etal* (1988). The formation of malondialdehyde is the basis for the well-known TBA method used for evaluating the extent of lipid peroxidation. At low pH of 2-3 and high temperature (100^{o}C), malondialdehyde (MDA) binds thiobarbituric acid (TBA) to form a pink complex which absorbs maximally at 532 nm. The measurement of MDA is an indirect method for assessing the extent of lipid peroxidation.

Principle: At low pH of 2-3 and high temperature (100^{0}C), malondialdehyde (MDA) binds thiobarbituric acid (TBA) to form a pink complex which absorbs maximally at 532 nm.

*Procedure:*To 0.5ml of tissue homogenate, 0.5ml saline and 1.0ml of 105% trichloroacetic acid (TCA) was added. Exactly 0.25ml of 0.1M TBA was then added to the mixture. The mixture was incubated for 1 hour at 95^{0}C, cooled and centrifuged at 3000 rpm for 20 min. The absorbance of the pink colour produced was read at 535nm.

3.2.18Isolation of components from fraction 6 using thin layer chromatography (TLC)

Thin layer chromatography (TLC) was carried out to isolate the principle components that were present in fraction 6 of most effective extracts of plant using the method of Harborne, (1998). Possible components were detected and identified from fraction 6(F6) using thin layer chromatography (TLC). Commercially prepared TLC aluminium sheets of 20 x 20cm lined with silica gel were used. The plate was cut to size of 5 x 10cm. Fraction 6 (F6) of 10ml aliquot was collected into the beaker. The solvent was allowed to evaporate and the content spotted at the bottom of the TLC plate (about 0.5cm from the base) using a micro haematocrit capillary tube until the quantity loaded was adjudged sufficient for the experiment. The plates were placed in a chromatographic tank and eluted with a mixture of different solvents(methanol, ethanol, ethylacetate, n-hexane, acetic acid), with hexane/ethyl

acetate giving the best resolution. Hexane / ethyl acetate at different ratios (100% hexane, 9:1 v/v, 8:2 v/v, 7:3 v/v, 6:4 v/v, 5:5 v/v, 4:6 v/v, 3:7v/v, 2:8 v/v, 1:9 v/v, 100% ethyl acetate) were used. Thereafter the plates were removed, air-dried and developed by spraying with 20% sulphuric acid in methanol. They were then viewed under UV light. Spots were seen. The Rf value was calculated as follows:

$$Rf = \frac{\text{Distance moved by the components}}{\text{Diistance moved by the solvent front}}$$

3.2.19Determination of hyperlipidemic effect of the fraction 6 TLC components

3.2.19.1Animal grouping and treatment

The rats were divided into 7 groups of 4 rats each and the induction lasted for three days after they were confirmed to be hyperlipidemic as described in section 3.2.6.1. Atorvastatin was administered orally as described in section 3.2.7. At the end of the three days treatment orally using the feeding tube for three days was terminated and the sample was collected as described in section 3.2.8. The supernatant (serum) collected was then subjected to biochemical analysis as described in section 3.2.9.

Group1: Normal rats administered distilled water and feed only (normal control).

Group2: Hyperlipidemic rats, administered distilled water and feed only (hyperlipidemic control).

Group4: Hyperlipidemic rats, administered 20mg/kg body weight of fraction 6

Group5: Hyperlipidemic rats, administered 20mg/kg body weight component 1 on TLC of Hexane /Ethylacetate 7:3

Group6: Hyperlipidemic rats, administered 20mg/kg body weight of component 2 on TLC of Hexane /Ethylacetate 7:3

Group7: Hyperlipidemic rats, administered 10 mg/kg body weight of Atorvastatin.

3.2.20Fourier transform infra-red spectroscopy analysis of the components

The FTIR analysis of fraction 6 and the two sub fractions was carried out to determine functional group(s) present using FTIR spectrophotometer (Shidmazu model) (Takahama and Dillner,2015), available at the National Research Institute for Chemical Technology (NARICT) Laboratory Zaria. FT-IR stands for Fourier Transform InfraRed, the preferred method of infrared spectroscopy. IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis.

All spectra were obtained with the aid of an OMNI-sampler attenuated total reflectance (ATR) accessory on a Nicolet FTIR spectrophotometer (Thermoscientific Nicolet is10, USA) followed by previous methods with some modifications (Ellis *et al*., 2002; Lu *et al*., 2004; Liu *et al*., 2006). A small amount of sample was respectively placed directly on the germanium piece of the infrared spectrometer with constant pressure applied and data of infrared absorbance, collected over the wave number ranged from 4000 cm^{-1} to 675 cm^{-1} and computerized for analyses by using the Omnic software (version 5.2). The reference spectra were acquired from the cleaned blank crystal prior to the presentation of each sample replicate. All spectra were collected with a resolution of 4-1 cm and to improve the signal-to-noise ratio, 256 scans were co-added and averaged. Samples were run in triplicate and all of them were undertaken within a day period.

3.2.21 Identification of active component by gas chromatography/mass spectroscopyof the components

Active components were identified from fraction 6 and the subfraction 1 and 2 using hyphenated separation techniques (gas chromatography coupled to mass spectrometry (GC-MS) composed of two major building blocks by the method of Sathya *et al*., (2010). The samples (1µl) was injected into a RTX-5 column of GC-MS. Helium, a gas that propelled the sample down the column was used as carrier gas at a flow rate 1.58 ml/min at 108 kpa inlet pressure. The prepared sample was injected into the injection port at one end of a metal column packed with substrate material. Temperature programming was maintained from 100°C to 200°C with constant rise of 5°C/min and then held isothermal at 200°C for 6 min; further the temperature was increased by 10 °C/min up to 290°C and again held isothermal at 290°C for 10min. The injector and ion source temperatures were 270 °C and 250°C, respectively. The samples (2mg/ml) dissolved in ethanol (HPLC grade, Merck, India) were injected. Mass spectra were taken at 70eV; a scan interval of 0.5s and fragments from 40 to 800Dalton. The molecules were retained by the column and then eluted (come off) from the column at different times (called the retention time), and this allowed the mass spectrometer downstream to capture, ionize, accelerate, deflect, once the sample was fragmented it was then detected, usually by an electron multiplier diode, which essentially turns the ionized mass fragment into an electrical signal and then detect the ionized molecules separately using a detector at the other end of the column. The difference in the chemical properties between different molecules of active ingredient(s) in a mixture was separated as the sample travels the length of the column, passed through the transfer line and entered into the mass spectrometer. They were ionized by various methods with

typically only one method being used at any given time. Interface temperature was kept at 25^0C. The ionization mode was electron impact ionization and the scanning range was from 45-450 (m/z). The final confirmation of constituents was made by computer matching of the mass spectra of peaks with the National Institute Science and Technology (NIST) libraries 2005 mass spectral database at the Shimadzu Training Centre for Analytical Instruments (STC) Lagos**.**

3.2.22 Statistical analysis

All the data or experimental values were expressed as the mean $\pm$ S.D. Statistical analysis was carried out using one way analysis of variance (ANOVA) as in standard statistical software package of social science (SPSS version 20). A "P" values less than 0.05 was considered to be statistically significant.

CHAPTER FOUR

4.0 Results

4.1 Qualitative Analysis on Phytochemical Constituents of Powdered Sample of Leaves and Stem of *Tephrosia vogelii* Hook. F.

Qualitative analysis on phytochemical constituents of powdered sample of leaves and stem of *T. vogelii* is shown in Table 4.1. The results revealed the presence of alkaloids, saponins, phlobatannins and flavonoids in powdered sample of leaves and stem of *T. vogelii*. Terpenes and anthaquuonones were only present in the leaves but absent in stem. Tannins were absent in both leaves and stem.

4.2 Percentage Yield of Aqueous, Acetic Acid, and Ethanol Extracts of the Leaves and Stem of *Tephrosia vogelii*

Percentage yield of aqueous, acetic acid and ethanol extracts of the leaves and stem of *Tephrosia vogelii* is presented in Table 4.2. The results revealed that the yield of the aqueous extract of the leaves was significantly ($p<0.05$)higher than other extracts. The yield of ethanol extract of the leaves and aqueous extract of the stem were not significant($p>0.05$) differentbut were significantly($p<0.05$) higher than the remaining extracts which intend recorded no significant ($p>0.05$) difference.

Table 4.1 Qualitative Analysis on Phytochemical Constituents of Powdered Sample of Leaves and Stem of *Tephrosia vogelii* Hook. F.

Phytochemical	LeavesPowder	StemPowder
Alkaloids	+	+
Saponins	+	+
Flavonoids	+	+
Phlobatannins	+	+
Terpenes and Steroids	+	–
Anthraquonones	+	–
Tannins	–	–

Key

+ = Present

- = Absent

Table 4.2: Percentage Yield of Aqueous, Acetic Acid, and Ethanol Extracts of Leaves and Stem of *Tephrosia vogelii*

Plant Parts	Extracts	Percentage Yield %
Leaves	Aqueous	18.21 ± 1.12^{c}
	Ethanol	12.24 ± 3.15^{b}
	Acetic Acid	9.13 ± 2.11^{a}
Stem	Aqueous	11.32 ± 4.23^{b}
	Ethanol	9.31 ± 2.04^{a}
	Acetic Acid	7.21 ± 1.01^{a}

Values are means ± SD of four determinations. Values with different superscripts in the same column are significantly different ($P<0.05$)

4.3 Effects of Aqueous, Ethanol, and Acetic Acid Extracts of*Tephrosia vogelii*Leaves Extracts on Lipid Profile in Poloxamer 407 and High Fat Diet Induced Hyperlipidemic Rats.

Effects of the aqueous, ethanol, and acetic acid extracts of*Tephrosia vogelii* leaves on poloxamer 407 and high fat diet induced hyperlipidemic rats are shown in Table 4.3. The results showed that both poloxamer and high fats diet generated significant($p<0.05$) increase in serum level of total cholesterol, triacylglycerol and LDL-cholesterol, and a significant ($p<0.05$) decrease in HDL-cholesterol, compared to the normal control. In the treated groups, there was a significant ($p<0.05$) decrease in total cholesterol, triacylglycerol and LDL-cholesterol when compared to the both poloxamer and high fats diet control groups. Also, there was a significant($p<0.05$) increase when compared with the normal control with the exception of triacylglycerol levels of the group treated with 50mg/kg of aqueous extract which recorded no significant difference ($p>0.05$) compared to the normal control. In HDL-cholesterol levels, group receiving aqueous extract recorded no significant ($p>0.05$) difference compared to the normal control while there is a significant increase ($p<0.05$) compared to the poloxamer and high fats diet control. The groups receiving acetic acid and ethanol recorded significant decrease in HDL-cholesterol compared to normal control; non-significant ($p>0.05$) difference compared to the poloxamer control and a significant ($p<0.05$) increase compared to high fats diet control. The result further indicate that poloxamer 407 gave the best induction of lipid. In hyperlipidemic rats, the three different extracts significantly lowered the lipid with the groups administered 50mg/kg aqueous extract showing the highest reduction of 49%.

Table 4.3: Effects of Aqueous, Ethanol, and Acetic Acid Extracts of *Tephrosia vogelii*Leaves Extracts on Lipid Profile in Poloxamer 407 and High Fat Diet InducedHyperlipidemic Rats

Group(n=4)	Serum TC (mg/dl)	Serum TAG (mg/dl)	Serum HDLc (mg/dl)	Serum LDL-c (mg/dl)
NC	112.09± 11.42^{b}	76.87± 14.19^{b}	27.32±4.87^{d}	80.22±12.35^{e}
HypChfd	201.06± 8.30^{d}	118.33±11.15^{e}	11.23± 5.31^{a}	101.21± 1.23^{f}
HypCpolox	251.04± 6.11^{e}	195.33±2.15^{f}	15.38± 4.20^{b}	141.01± 4.16^{g}
Hyppolox +Aq50	113.25±7.23^{b} (49%)	78.98±8.34^{b} (58%)	21.74± 3.14^{c}	45.43± 8.23^{b}
Hyppolox+AA50	130.72±0.20^{c} (41%)	95.19±15.34^{d} (50%)	16.62 ± 8.74^{b}	66.18± 27.58^{d}
Hyppolox+Eth50	124.23±11.90^{c} (44%)	87.80±25.95^{c} (54%)	18.04± 1.55^{b}	59.56± 8.33^{c}
Hyppolox +Std	81.23±7.51^{a} (63%)	60.54±7.23^{a} (68%)	45.23± 4.62^{e}	31.33± 1.45^{a}

Values are means ± SD of four determinations. Values with different superscripts in the same column are significantly different ($p<0.05$). Values in parenthesis indicate percentage reduction.

NC: Normal rats Control, HypChfd: Hyperlipidemic rats Control of high fat diet, HypCpolox: Hyperlipidemic rats Control of poloxamer 407, Hyppolox +Std: Hyperlipidemic rats of poloxamer 407 + Standard Drug (Atorvastatin10mg/kg), Hyppolox + Aq50: Hyperlipidemic rats of poloxamer 407 + Aqueous Extract (50mg/kg), Hyppolox +AA50: Hyperlipidemic rats of poloxamer 407 + Acetic acid Extract (50mg/kg), Hyppolox +Eth50: Hyperlipidemic rats of poloxamer 407 + Ethanolic Extract (50mg/kg).

TC: Total cholesterol, TAG: Triacylglycerol, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol,

4.4: Effects of Aqueous, Ethanol, and Acetic Acid of*Tephrosia vogelii* Stem Extracts on Lipid Profile in Poloxamer 407 and High Fat Diet Induced Hyperlipidemic Rats

The effects of the aqueous, ethanol, and acetic acid of *T. vogelii* stem extracts on poloxamer 407 and high fat diet induced hyperlipidemic rats are shown in Table 4.4. The result indicates that there was a significant ($p<0.05$) increase in total cholesterol, triacylglycerol and LDL-cholesterol and a significant ($p<0.05$) decrease in HDL-cholesterol in the poloxamer and high fats diet control groups, compatred to the normal control. In the groups treated with extract, there was significant ($p<0.05$) decrease in total cholesterol, triacylglycerol and LDL-cholesterol compared to the poloxamer and high fats diet control while a significant ($p<0.05$) increase was recorded compared to the normal control. In HDL-cholesterol levels, group receiving aqueous extract recorded non significant ($p>0.05$) difference compared to the normal control and a significant increase compared to the poloxamer and high fats diet controls while the group receiving acetic acid recorded a significant ($p<0.05$) decrease compared to normal control, significant ($p<0.05$)increase compared to high fats diet control and non significant ($p>0.05$) difference compared to poloxamer control. The group receiving ethylacetate extract recorded a significant ($p<0.05$) decrease in HDl-cholesterol compared to the normal control and a significant ($p<0.05$) increase compared to the high fats diet and poloxamer controls. Therefore in hyperlipidemic rats, the three different extracts significantly ($p<0.05$) lowered their lipid with the groups administered 50mg/kg aqueous extract showing the highest percentage reduction.

4.5: Quantitative Phytochemical Constituents of Aqueous Leaves Extract of *Tephrosia vogelii*

The quantitative analysis of phytochemical constituents of aqueous leaves extract of *T. vogelii* is presented in Figure 4.1.

4.6 Lethal Dose (LD_{50}) Determination of Aqueous Leaves Extract of *Tephrosia vogelii* in Albino Rats

The lethal dose determination of aqueous leaves extract of *T. vogelii* in albino rats for 48 hours is presented in Table 4.5. There was no mortality within 48 hours after oral administration of 10, 100 and 1000 mg/kg body weight of aqueous leaf extract to albino rats in the first phase. However, upon oral administration of 1000, 1600, 2900 and 5000 mg/kg body weight of the extract to a different set of albino rats in the second phase, there was 100% mortality in the group administered 2900 mg/kg and 5000 mg/kg body weight within 48 hours. Thus, the lowest lethal dose was taken as 2900 mg/kg body weight and the highest non- lethal dose was 1600mg/kg body weight.

Therefore, the oral LD_{50} value was calculated as the square root of the products of the highest non-lethal dose and the lowest lethal dose to be 2154mg/kg body weight.

Table 4.4: Effects of Aqueous, Ethanol, and Acetic Acid of *Tephrosia vogelii* Stem Extracts on Lipid Profile in Poloxamer 407 and High Fat Diet Induced Hyperlipidemic Rats

Groups	Serum TC (mg/dl)	Serum TAG (mg/dl)	Serum HDLc (mg/dl)	Serum LDL-c (mg/dl)
NC	112.09± 11.42^{b}	76.87± 14.19^{b}	27.32±4.87^{d}	80.22±12.35^{a}
HypChfd	201.06± 8.30^{e}	118.33±11.15^{d}	11.23± 5.31^{a}	101.21± 1.23^{e}
HypCpolox	251.04± 6.11^{f}	195.33±2.15^{e}	15.38± 4.20^{b}	141.01± 4.16^{g}
Hyppolox +Aq50	123.13±4.12^{c} (44%)	101.76±6.27^{c} (46%)	23.63± 2.10^{d}	58.51± 2.31^{b}
Hyppolox+AA50	137.65±31.52^{d} (38%)	119.11±21.41^{d} (37%)	16.79 ± 9.65^{b}	71.23± 35.76^{d}
Hyppolox+Eth50	131.31±22.78^{d} (41%)	110.21±34.84^{d} (42%)	20.11± 2.42^{c}	62.34± 7.41^{c}
Hyppolox +Std	81.23±7.51^{a} (63%)	60.54±7.23^{a} (68%)	45.23± 4.62^{e}	31.33± 1.45^{a}

Values are means ± SD of four determinations. Values with different superscripts in the same column are significantly different ($p<0.05$)

NC: Normal rats Control, HypChfd: Hyperlipidemic rats Control of high fat diet, HypCpolox: Hyperlipidemic rats Control of poloxamer 407, Hyppolox +Std: Hyperlipidemic rats of poloxamer 407 + Standard Drug (Atorvastatin10mg/kg), Hyppolox + Aq50: Hyperlipidemic rats of poloxamer 407 + Aqueous Extract (50mg/kg), Hyppolox +AA50: Hyperlipidemic rats of poloxamer 407 + Acetic acid Extract (50mg/kg), Hyppolox +Eth50: Hyperlipidemic rats of poloxamer 407 + Ethanolic Extract (50mg/kg).

TC: Total cholesterol, TAG: Triacylglycerol, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol,

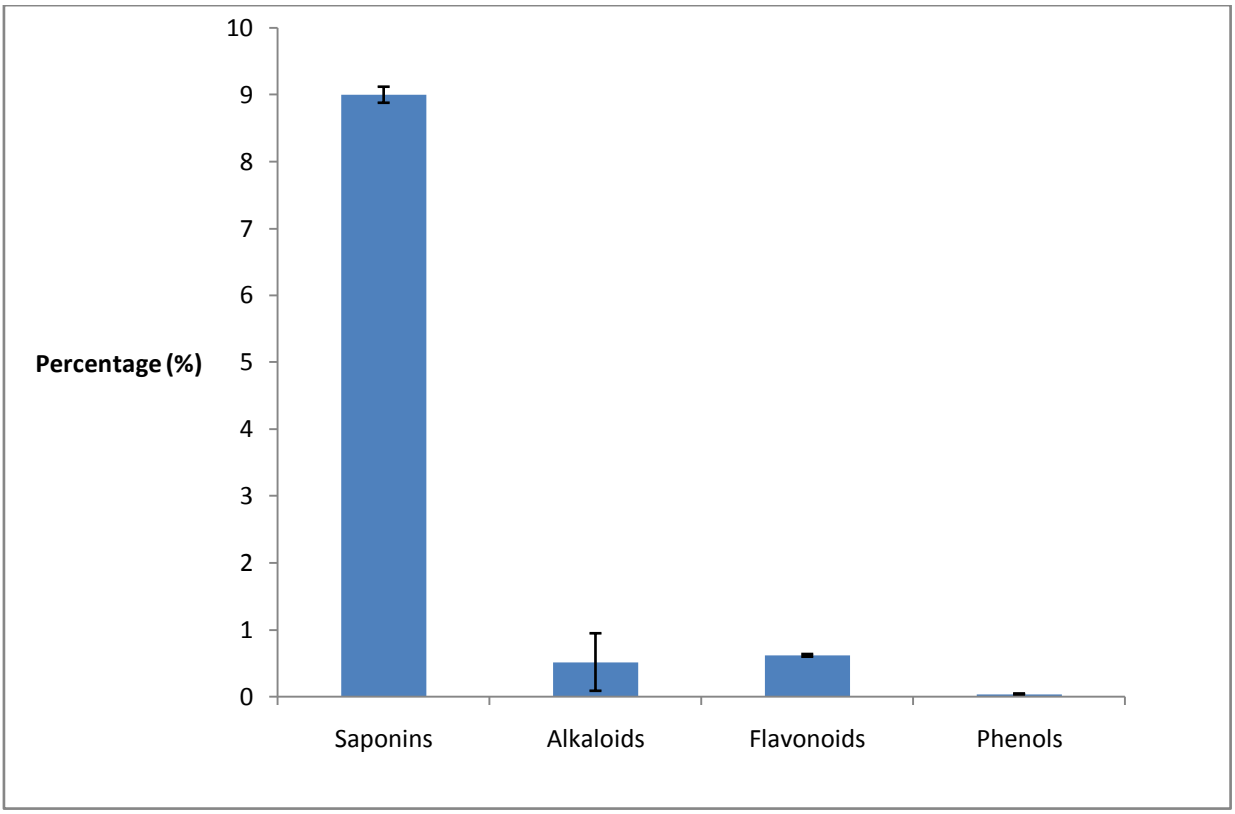


Figure 4.1: Quantitative Phytochemical constituents of Aqueous Leaves Extract of *Tephrosia vogelii*

Table 4.5: Lethal Dose (LD_{50}) Determination of Aqueous Leaves Extract of *Tephrosia vogelii* In Albino Rats

First phase	Dose (mg/kg)	Number of animals	Number of death after 48 hrs	% Mortality
Group 1	10	3	0	0
Group 2	100	3	0	0
Group 3	1000	3	0	0
Second phase				
Group 1	1000	1	0	0
Group 2	1600	1	0	0
Group 3	2900	1	1	100
Group 4	5000	1	1	100

$LD_{50} = \sqrt{}$ Highest non-lethal dose x Lowest lethal dose
Highest non-Lethal dose =1600mg/Kg body eight
Lowest lethal dose was = 2900 mg/kg body weight
$LD_{50} = \sqrt{1600 \times 2900}$
LD_{50} = 2154mg/kg body weight

4.7: Effects of Ten Fractions (F1-F10) of *Tephrosia vogelii* Aqueous Leaves Extracts on Lipid Profile in Poloxamer 407 Induced Hyperlipidemic Rats

Ten fractions (F1-F10) of *T. vogelii* leaves on poloxamer 407 induced hyperlipidemic rats(Table 4.6) revealed that the fraction 6 (F6) significantly ($p<0.05$) lowered the TC and TAG when compared to other fractions. Administration of fraction 6 significantly ($p<0.05$) increased HDL when compared with other fractions, but significantly ($p<0.05$) lower than the animal in the normal control group.

4.8: Percentage yields and Phytochemical Constituents of Fraction 6 (F6) of *Tephrosia vogelii* Aqueous Leaves Extracts

Percentage yields of the ten fractions and qualitative phytochemical constituent offraction 6 of aqueous leaves are shown in Table 4.7 and Table 4.8 respectively. Percentage yield showed that all fractions had similar yields of 3% with fraction 3 recording the highest yield (3.32). Fraction1 and 4 recording the least of 3.20%. The total percentage yield was 32.56% (Table 4.7). Resultsof phytochemical content showed the presence of alkaloids, flavonoids and saponins. While tannins, phlobatannins, anthraquinones, terpenes and steroids were absent (Table 4.8).

4.9: Effects of *Tephrosia vogelii* Aqueous Leaves Extract and Fraction 6 (F6) on Serum Lipid Profile in Poloxamer 407 Induced

Aqueous leaves extract and fraction 6 of *T. vogelii* on poloxamer 407 induced hyperlipidemic rats in Table 4.9 revealed that the fraction 6 (F6) significantly ($p<0.05$) lowered the TC, TAG and LDL when compared to aqueous leaves extract, normal and

poloxamer controls. Administration of fraction 6 significantly ($p<0.05$) increased HDL when compared to aqueous leaves extract and poloxamer control, but significantly ($p<0.05$) lower than the animal in the normal control group.

Table 4.6: Effects of Ten Fractions (F1-F10) of *Tephrosia vogelii* Aqueous Leaves Extracts on Lipid Profile in Poloxamer 407

Groups	Serum TC (mg/dl)	SerumTAG (mg/dl)	Serum HDL-c (mg/dl)	Serum LDL-c (mg/dl)
NC	116.09± 11.42^{h}	79.87± 15.19^{g}	27.39±4.87^{g}	82.32±12.35^{f}
HyperC	235.06± 8.30^{i}	189.34±02.15^{i}	15.40± 4.21^{b}	140.01± 3.15^{g}
HypCpolox + F1 50mg/kgbodyweight	111.05± 2.31^{d}	77.33±13.26^{e}	11.49± 2.21^{a}	44.21± 7.14^{c}
HypCpolox + F2 50mg/kgbodyweight	112.25± 7.23^{e}	76.98± 8.34^{d}	21.74± 3.14^{c}	43.47± 6.24^{c}
HypCpolox + F3 50mg/kgbodyweight	111.72±0.20^{d}	75.19±15.34^{c}	22.62 ±8.74^{c}	42.18± 27.58^{b}
HypCpolox + F4 50mg/kgbodyweight	114.23±11.90^{e}	79.80± 25.95^{g}	20.04±1.55^{b}	46.56±8.33^{e}
HypCpolox + F5 50mg/kgbodyweight	113.10 ±8.20^{f}	78.13±6.90^{f}	24.51±3.14^{e}	43.70±3.10^{c}
HypCpolox + F6 50mg/kgbodyweight	99.42± 9.57^{b}	71.98± 9.46^{b}	26.79± 3.10^{f}	41.62± 6.90^{b}
HypCpolox + F7 50mg/kgbodyweight	110.31± 7.24^{c}	77.54± 9.23^{e}	23.77±4.12^{d}	44.43± 1.40^{c}
HypCpolox + F8 50mg/kgbodyweight	113.32± 8.48^{f}	70.66± 8.12^{b}	25.18± 5.17^{f}	48.18± 5.30^{c}
HypCpolox + F9 50mg/kgbodyweight	112.11±5.12^{e}	77.22± 2.13^{e}	27.23± 9.21^{g}	44.22± 1.23^{c}
HypCpolox + F10 50mg/kgbodyweight	110.44± 6.13^{c}	85.71± 9.20^{h}	29.70± 3.12^{h}	45.21± 3.40^{d}
Hyppolox + STD	81.23± 7.51^{a}	60.54± 7.23^{a}	45.23± 4.62^{i}	31.33± 1.45^{a}

Values are means ± SD of four determinations. Values with different superscripts in the same column are significantly different ($p<0.05$)

NC: Normal rats Control, HypCpolox: Hyperlipidemic rats Control of poloxamer 407, Hyppolox +Std: Hyperlipidemic rats of poloxamer 407 + Standard Drug (Atorvastatin), Hyppolox + Aq50: Hyperlipidemic rats of poloxamer 407 + Aqueous Extract (50mg/kg), Hyppolox +AA50: Hyperlipidemic rats of poloxamer 407 + Acetic acid Extract (50mg/kg), Hyppolox +Eth50: Hyperlipidemic rats of poloxamer 407 + Ethanolic Extract (50mg/kg). TC: Total cholesterol, TAG: Triacylglycerol, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol,

Table 4.7 Percentage Yields of Fractions of Aqueous Leaves Extact of *Tephrosia vogelii*

Fractions	Weight(g)	%Yield
F1	0.160	3.20
F2	0.165	3.30
F3	0.166	3.32
F4	0.161	3.22
F5	0.163	3.26
F6	0.160	3.20
F7	0.162	3.24
F8	0.164	3.28
F9	0.165	3.30
F10	0.162	3.24
Total	1.628	32.56

Table 4.8: Some Phytochemical Constituents of Fraction 6 (F6) of Leaves of *Tephrosia vogelii*

Phytochemicals	Fraction 6 (F6) Leaves
Alkaloids	+
Saponins	+
Flavonoids	+
Tannins	_
Phlobatannins	_
Terpenes and Steroids	_
Anthraquinones	_

Key

+ = Present

- = Absent

Table 4.9: Effects of *Tephrosia vogelii* Aqueous Leaves Extract and Fraction 6 (F6) on Serum Lipid Profile in Poloxamer 407 Induced Hyperlipidemic Rats

Groups	Serum TC (mg/dl)	Serum TAG (mg/dl)	SerumLDL-c (mg/dl)	Serum HDL-c (mg/dl)
NC	115.03± 23.11^{c}	79.32± 12.67^{b}	81.12±3.10^{d}	30.12±11.12^{d}
HyperC	232.06± 5.21^{f}	199.24±11.15^{e}	142.38± 4.20^{e}	16.01± 4.16^{a}
Hyper+Aq100	120.75± 21.72^{e}	87.29±15.35^{d}	50.52 ± 7.74^{c}	17.07±03.98^{a}
Hyper+Aq200	117.17±10.89^{d}	85.90± 15.95^{c}	51.03± 1.98^{c}	18.56± 7.25^{b}
Hyper+F(F6)100	98.04± 7.21^{a}	71.24±32.10^{a}	40.21± 1.31^{b}	25.10± 2.12^{c}
Hyper+ F(F6)200	97.05± 4.21^{a}	69.11±13.21^{a}	39.41± 3.12^{b}	26.01± 5.17^{c}
Hyper+Std	99.29±2.66^{b}	69.87± 5.41^{a}	37.12± 1.21^{a}	32.12± 2.17^{e}

Values are means ± SD of four determinations. Values with different superscripts in the same column are significantly different ($p<0.05$).

NC: Normal rats Control, HyperC: Hyperlipidemic rats Control, Hyper+Std: Hyperlipidemic rats + Standard Drug (Atorvastatin 10mg/kg), Hyper+Aq100: Hyperlipidemic rats + Aqueous Extract (100mg/kg), Hyper+Aq200: Hyperlipidemic rats + Aqueous Extract (200mg/kg), Hyper+ F(F6)100: Hyperlipidemic rats + fraction 6 (100mg/kg), Hyper+ F(F6)200: Hyperlipidemic rats + fraction 6 (200mg/kg).

TC: Total cholesterol, TAG: Triacylglycerol, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol,

4.10: Effects of *Tephrosia vogelii* Aqueous Leaves Extract and Fraction 6 (F6) on Atherogenic Predictor Indices of P407 Induced Hyperlipidemic and Normal Rats

The effects of daily oral administration of *T. vogelii* aqueous leaves extract and fraction 6 (F6) in poloxamer (407) induced hyperlipidemic rats on value of serum atherogenic risk predictor indices is presented in Table 4.10. The results showed that hyperlipidemic controlhad significant ($p< 0.05$) decrease in HDL-C/TC ratio and a significant ($p<0.05$) increase in LDL-C/HDL-C and LOG (TG/HDL-C) ratio when compared with all other groups. Animals induced and treated show that the aqueous leaves extract and fraction 6 (F6) significantly ($P<0.05$) increased the HDL-C/TC and decreased the LDL-C/HDL-C and LOG (TG/HDL-C) ratios when compared to the hyperlipidemic not treated group. When compared to the normal control,group receiving 100mg/Kg of aqueous extract had a significant ($p<0.05$) decrease in HDL-C/TC, significant increase ($p<0.05$) in LDL-C/HDL-C and no significant difference ($p>0.05$) in LOG (TG/HDL-C) while group receiving 200mg/kg of aqueous extract recorded no significant ($p>0.05$) difference in HDL-C/TC and significant ($p<0.05$) decrease in LDL-C/HDL-C and LOG (TG/HDL-C). Group receiving 100 mg/Kg of F6 recorded no significant ($P>0.05$) difference in HDL-C/TC while the

group receiving 200mg/Kg recorded a significant ($P<0.05$) increase compared to the normal control. Groups receiving F6 recorded significant ($p<0.05$) decrease in LDL-C/HDL-C and LOG (TG/HDL-C) compared to normal control.

4. 11: Effects of *Tephrosia vogelii* Aqueous Leaves Extract and Fraction 6 (F6)on Kidney Function Parameters of P407 Induced Hyperlipidemic and Normal Rats

Creatinineand urea concentrations in the serum of poloxamer (407) induced hyperlipidemic and normal rats are presented in Table 4.11. The results showed thatcreatinine and urea concentrationsignificantly ($p<0.05$) increased in hyperlipidemic control when compared with all other groups. The aqueous leaves extract and fraction 6 (F6) significantly ($p<0.05$) decreased the creatinine and urea concentration when compared to hyperlipidemic control group and significant ($p<0.05$) increase was recorded when compared to the normal control group.

Table 4.10: Effects of *Tephrosia vogelii* Aqueous Leaves Extract and Fraction 6 (F6) on
Atherogenic Predictor Indices of P407 Induced Hyperlipidemic and Normal Rats

Groups	HDL-c/ TC	LDL-c/HDL-c	LOG(TAG /HDL-c)
NC	0.261± 0.001^{c}	2.700±0.010^{e}	0.238± 0.006^{e}
HyperC	0.069± 0.003^{a}	8.875± 0.020^{g}	0.664±0.015^{f}
Hyper+Aq100	0.141± 0.002^{b}	2.941± 0.001^{f}	0.232±0.011^{e}
Hyper+Aq200	0.153±0.009^{c}	2.833± 0.004^{d}	0.138± 0.020^{d}
Hyper+F(F6)100	0.200± 0.001^{c}	1.600± 0.021^{c}	0.096±0.010^{c}
Hyper+ F(F6)200	0.268± 0.011^{d}	1.500± 0.002^{b}	0.049±0.012^{b}
Hyper+Std	0.323±0.006^{e}	1.156± 0.004^{a}	0.008± 0.001^{a}

Values are means ± SD of four determinations. Values with different superscripts in the same column are significantly different ($p<0.05$).

NC: Normal rats Control, HyperC: Hyperlipidemic rats Control, Hyper+Std: Hyperlipidemic rats + Standard Drug (Atorvastatin 10mg/kg), Hyper+Aq100: Hyperlipidemic rats + Aqueous Extract (100mg/kg), Hyper+Aq200: Hyperlipidemic rats + Aqueous Extract (200mg/kg), Hyper+ F(F6)100: Hyperlipidemic rats + fraction 6 (100mg/kg), Hyper+ F(F6)200: Hyperlipidemic rats + fraction 6 (200mg/kg).

TC: Total cholesterol, TAG: Triacylglycerol, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol,

Table 4.11: Effects of *Tephrosia vogelii* Aqueous Leaves Extract and Fraction 6 (F6)on Kidney Function Parameters of P407 Induced Hyperlipidemic and Normal Rats.

Groups	Creatinine (mg/dl)	Urea (mg/dl)
NC	3.61 ± 0.04 [a]	72.64 ± 1.43 [a]
HyperC	10.82 ±1.13 [f]	162.47 ± 3.32 [f]
Hyper+Aq100	7.51 ± 0.14 [e]	121.07 ± 0.21 [e]
Hyper+Aq200	6.12 ± 1.01 [d]	104.45 ± 1.36 [d]
Hyper+F(F6)100	5.42 ± 1.13 [c]	98.06 ± 1.43 [c]
Hyper+ F(F6)200	4.89 ± 0.20 [b]	87.98 ± 0.60 [b]
Hyper+Std	3.21 ± 0.02[a]	71.61 ± 1.71[a]

Values are means ± SD of four determinations. Values with different superscripts in the same column are significantly different ($p<0.05$).

NC: Normal rats Control, HyperC: Hyperlipidemic rats Control, Hyper+Std: Hyperlipidemic rats + Standard Drug (Atorvastatin 10mg/kg), Hyper+Aq100: Hyperlipidemic rats + Aqueous Extract (100mg/kg), Hyper+Aq200: Hyperlipidemic rats + Aqueous Extract (200mg/kg), Hyper+ F(F6)100: Hyperlipidemic rats + fraction 6 (100mg/kg), Hyper+ F(F6)200: Hyperlipidemic rats + fraction 6 (200mg/kg).rats + fraction 6 (200mg/kg).

4.12: Effects of *Tephrosia vogelii* Aqueous Leaves Extract and Fraction 6 (F6) on Serum Biochemical Profile in Poloxamer- 407 Induced Hyperlipidemic Rats

Serumaspartate aminotransferase (AST), Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities(Figure 4.2) and total bilirubin and direct bilirubin (Figure 4.3) significantly ($p<0.05$) increased in the hyperlipidemic control groupwhen compared with normal control and treated groups. The serum ALT, ALP and TBwere significantly ($p<0.05$) higher in all the extract treated groups when compared with normal control. Fraction 6 and standard drug show significantly ($p<0.05$) lower AST and ALP when compared to normal control.

4.13: Antioxidant Effect of Aqueous Leaves and Fraction 6 (F6) of *Tephrosia vogelii* in Liver and kidney of rats induced Poloxamer- 407 Hyperlipidemia

The effect of aqueous extract and fraction 6 of *Tephrosia vogelii* on antioxidant parameters in the liver and kidney of rats is presented in Table 4.12 and Table 4.13 respectively. The results showed that hyperlipidemia significantly ($p<0.05$) lowered the SOD and CAT and significantly ($p<0.05$) increased GSH and MDA in rats liver and kidney when compared with rats in control group.

In the liver, all the treated groups showed significant ($p<0.05$) decrease in the level of SOD when compared with normal control with the exception of group receiving 100mg/kg of fraction 6 which recorded no significant($p>0.05$) difference. CAT in the treated groups were significantly ($p<0.05$) lower when compared with normal control, while MDA and GSH the liver were significant ($p<0.05$) higher when compared with normal control. When the treated groups were compared to the poloxamer control, there was significant ($p<0.05$) increase in SOD and CAT and significant ($p<0.05$) decreased in MDA and GSH (Table 4.12)

In the kidney, there was significant ($P<0.05$) decrease in the groups administered aqueous extract in SOD and no significant ($P>0.05$) change in the groups administered fraction 6 when compared to the normal control. There was significant ($p<0.05$) decrease in CAT in the extract treated group compared to normal control with the exception of CAT of group receiving 100mg/kg of fraction 6 which recorded no significant ($P>0.05$) difference. Significant ($p<0.05$) increase in GSH was recorded in all extract treated groups compare to normal control. For MDA level, with respect to normal control, significant ($P<0.05$) increase was recorded for group receiving 100mg/kg of aqueous extract and non significant ($P<0.05$) difference was obtained for group receiving 200mg/kg of aqueous extract while group receiving fraction 6 recorded significant ($p<0.05$) decrease in MDA. When compared to the hyperlipidemic control there was significant ($P<0.05$) increase in SOD and significant ($P<0.05$) decrease in GSH and MDA of treated groups. For CAT, non significant ($p>0.05$) difference was recorded in the extract and fraction treated group compared to the infected not treated with the exception of group receiving 100mg/kg of fraction 6 which recorded significant ($p<0.05$) increase (Table 4.13).

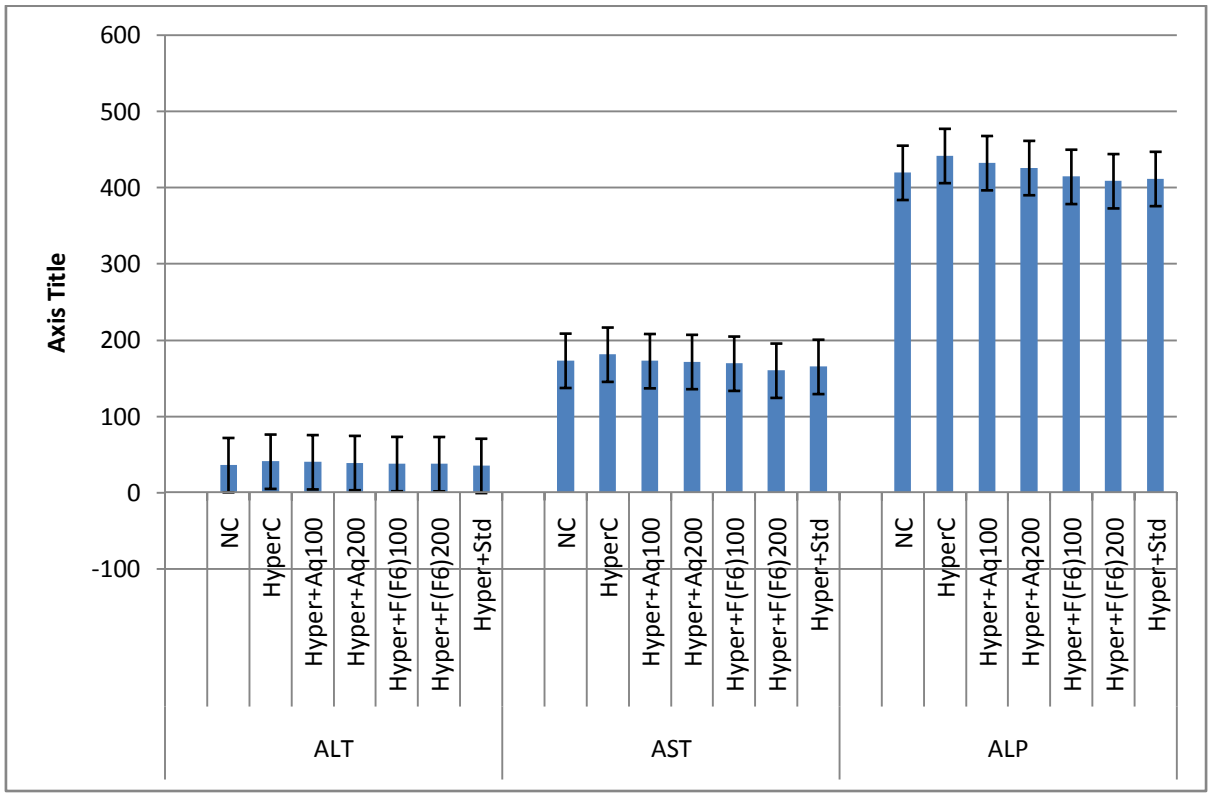


Figure 4.2: Effects of *T. vogelii* Aqueous Leaves Extract and Fraction 6 (F6) on Serum Biochemical Profile in Poloxamer- 407 Induced Hyperlipidemic Rats.

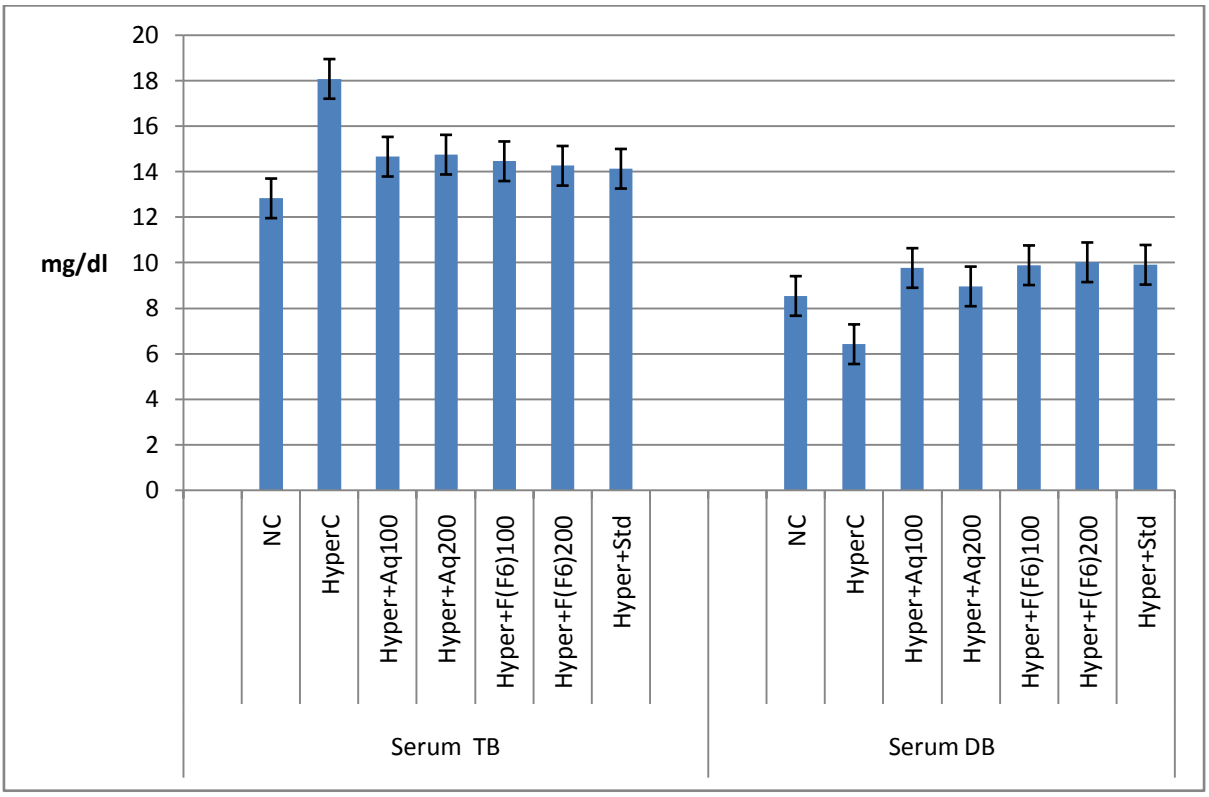


Figure 4.3: Effects of *Tephrosia vogelii* Aqueous Leaves Extract and Fraction 6 (F6) on Serum Total Bilirubin and Direct Bilirubin in Poloxamer- 407 Induced Hyperlipidemic Rats

Table 4.12: Antioxidant Effect of Aqueous Leaves and Fraction 6 (F6) of *Tephrosia vogelii* In the Liver of Hyperlipidemic Rats

Groups	SOD (U/I/I)	CAT(U/I/I)	GSH(umol/l)	MDA (umol/l)
NC	63.53± 1.67^{c}	3.52± 0.13^{d}	9.80±1.35^{a}	5.14± 0.76^{a}
HyperC	46.63± 6.06^{a}	1.34± 0.16^{a}	15.05± 1.74^{d}	8.90± 1.62^{d}
Hyper+Aq100	50.88± 3.58^{b}	1.48± 6.12^{a}	11.63± 3.16^{b}	7.50 ± 1.78^{c}
Hyper+Aq200	53.34± 6.89^{b}	1.51± 7.23^{b}	11.72± 3.39^{b}	7.12± 2.45^{c}
Hyper+ F(F6)100	52.34± 1.26^{b}	1.63± 2.31^{b}	12.34± 1.31b^{c}	6.91 ± 2.63^{b}
Hyper+ F(F6)200	60.12± 4.21^{c}	1.91± 4.41^{c}	13.61± 2.21^{c}	6.62± 2.29^{b}
Hyper+Std	56.72± 3.94^{b}	1.87± 0.49^{c}	11.10± 1.86^{b}	6.24± 1.66^{b}

Values are means ± SD of four determinations. Values with different superscripts in the same column are significantly different ($p<0.05$).

NC: Normal rats Control, HyperC: Hyperlipidemic rats Control, Hyper+Std: Hyperlipidemic rats + Standard Drug (Atorvastatin), Hyper+Aq100: Hyperlipidemic rats + Aqueous Extract (100mg/kg), Hyper+Aq200: Hyperlipidemic rats + Aqueous Extract (200mg/kg), Hyper+ F(F6)100: Hyperlipidemic rats + fraction 6 (100mg/kg), Hyper+ F(F6)200: Hyperlipidemic rats + fraction 6 (200mg/kg).

Table 4.13: Antioxidant Effect of Aqueous Leaves and Fraction 6 (F6) of *Tephrosia vogelii* In the Kidney of Hyperlipidemic Rats

Groups	SOD(U/I/I)	CAT(U/I/I)	GSH (umol/l)	MDA (umol/l)
NC	54.30± 0.94^{d}	2.24± 0.49^{b}	9.80±1.35^{a}	8.60±0.97^{c}
HyperC	38.34± 2.06^{a}	1.11± 0.10^{a}	15.05± 1.74^{f}	10.61± 0.22^{e}
Hyper+Aq100	44.18± 3.12^{b}	1.30± 6.12^{a}	14.54± 2.16^{e}	9.20 ±08.28^{d}
Hyper+Aq200	48.10± 6.13^{c}	1.50± 7.14^{a}	12.72± 1.39^{d}	8.53±14.25^{c}
Hyper+ F(F6)100	53.23± 3.62^{d}	1.78± 6.12^{a}	11.63± 3.23^{c}	7.02 ± 1.78^{b}
Hyper+ F(F6)200	56.34± 6.89^{d}	2.11± 3.19^{b}	11.12± 5.39^{c}	6.83± 24.45^{a}
Hyper+Std	51.50± 1.01^{d}	2.04± 0.36^{b}	10.10± 1.86^{b}	7.64± 1.20^{b}

Values are means ± SD of four determinations. Values with different superscripts in the same column are significantly different ($p<0.05$).

NC: Normal rats Control, HyperC: Hyperlipidemic rats Control, Hyper+Std: Hyperlipidemic rats + Standard Drug (Atorvastatin 10mg/kg), Hyper+Aq100: Hyperlipidemic rats + Aqueous Extract (100mg/kg), Hyper+Aq200: Hyperlipidemic rats + Aqueous Extract (200mg/kg), Hyper+ F(F6)100: Hyperlipidemic rats + fraction 6 (100mg/kg), Hyper+ F(F6)200: Hyperlipidemic rats + fraction 6 (200mg/kg).

4.14 Partial Purification of fraction 6 of aqueous leaves extract of *Tephrosia vogelii* usingTLC techniques

The results of thin layer chromatography (TLC)of fraction 6 are presented in figure 4.4, 4.5 and Table 4.14. Different solvent systems (methanol, ethanol, acetic acid, aqueous, hexane and ethylacetate at different ratios 100%, 9:1 v/v, 8:2 v/v, 7:3 v/v, 6:4 v/v, 5:5 v/v, 4:6 v/v, 3:7v/v, 2:8 v/v, 1:9 v/v, 100%) were used with hexane / ethyl acetate at the ratio 7:3 gave the clear fractions .Hexane / ethyl acetate at the ratio 7:3 expressed two (2) bands with the $R_{F\ values}$of 0.22 and 0.60.

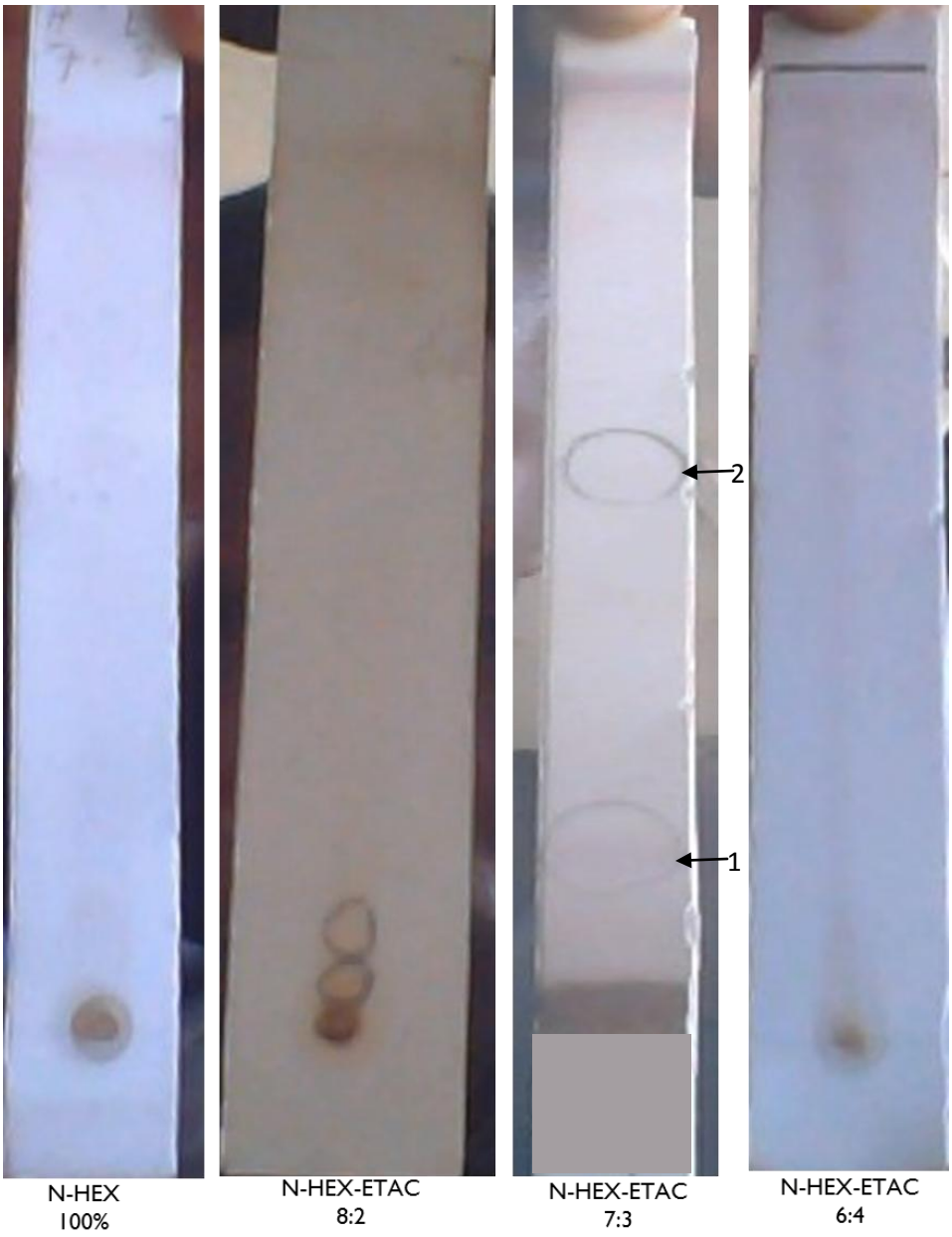


Figure 4.4: Prep-TLC of Bioactive fraction 6 of aqueous leaves extract of *Tephrosia vogelii*

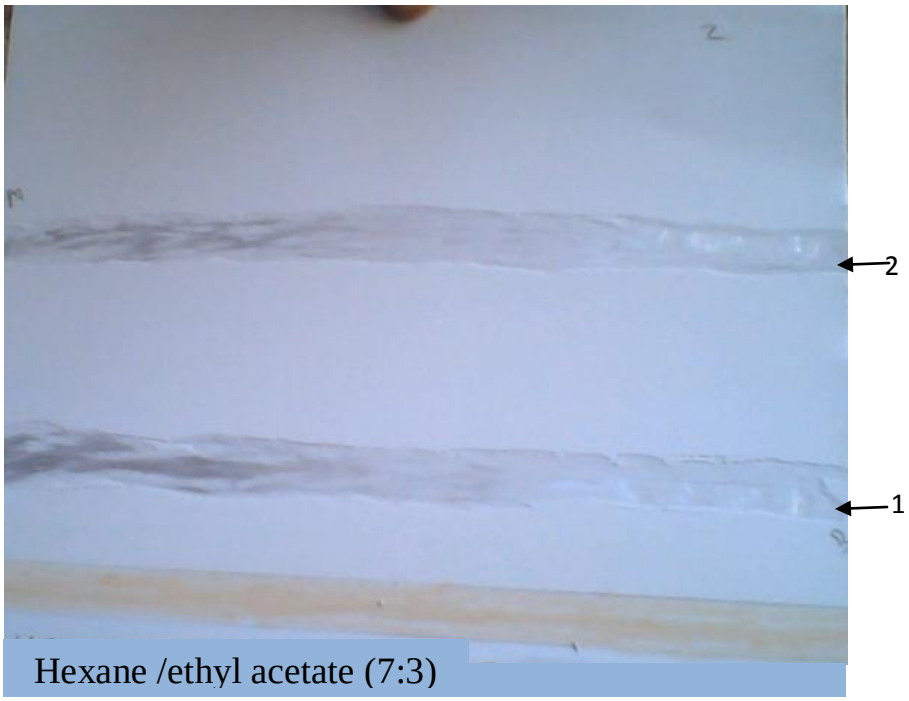


Figure 4.5: TLC Chromatogram of the subfractions

Table 4.14 Partial Purification of fraction 6 of aqueous leaves extract of *Tephrosia vogelii* by TLC techniques

Solvent system	Ratio of solvent system	No of fraction	R f values	Weight(g)	% yied
Hexane	100%	0	0.00		
Hexane/Ethyl acetate	9:1	2	$Rf_1$0.01, $Rf_2$0.05		
Hexane/Ethyl acetate	8:2	2	$Rf_1$0.06 $Rf_2$0.13		
Hexane/Ethyl acetate	7:3	2	$Rf_1$0.22	0.04	25.00
			$Rf_2$0.60	0.03	18.75
Hexane/Ethyl acetate	6:4	0	0.00		
Hexane/Ethyl acetate	5:5	0	0.00		
Hexane/Ethyl acetate	4:6	0	0.00		
Hexane/Ethyl acetate	3:7	0	0.00		
Hexane/Ethyl acetate	2:8	0	0.00		
Hexane/Ethyl acetate	1:9	0	0.00		
Ethyl acetate	100%	0	0.00		

4.15: Effects of subfractionswith Rf values (0.22 and 0.60) of fraction 6 of aqueous leaves extract of *Tephrosia vogelii* on Serum Lipid Profile in Poloxamer 407

Antihyperlipidemic effect of subfraction1 (Rf value 0.22)Table 4.15 significantly ($p<0.05$) reduced triglycerol (TG) when compared to animal treated with other *Tephrosia vogelii*fractions and show no significant ($p>0.05$) change when compared with standard drug and animal in control group. There was no significant ($p>0.05$) change in the level of total cholesterol (TC), low density lipoprotein (LDL),and high density lipoprotein (HDL) in *Tephrosia vogelii*fractionswhen compared with hyperlipidemic controlgroup.

4. 16 Characterization of the Antihyperlipidemic Compounds in the Bioactive Fraction by FTIR and GC-MS Spectroscopy

Fourier Transform Infrared(FTIR) Spectroscopy of fraction 6, subfraction 1 and 2 revealedten, eight and six functional groups respectively (Table 4.16a, 4.16b and 4.16c respectively) while the spectra are shown in appendix 3 to 5. In Table 4.16a, the peaks at around 1118.75and 1072.46 indicate the presence ofO-H, =C-O-C bond of medium intensity alcohols, ethers, phenolsand its derivative group. The peak at around 1419.66 indicates N-ONitro Compounds presence with strong intensity. The peak at around 3417.98 indicates the presence of C=O i.e. Carboxylic Acids compounds bond of strong intensity

group. The peak at around 401.21 and 1342.5 indicate the presence of C-I and C-F ie (Alkyl halides) bond of very strong intensity, spectrum.

On the other hand in Table 4. 16b and 4.16c, the peaks at 447.5, 609.53 and 848.71 indicate the presence of C-I, C-Br, C-Clie (Alkyl halide group) bond of strong intensity. While the peaks at 2376.38 and 2306.94 indicate the presence of C≡N bond of strong intensity, nitriles group present. Peak 1651.12 indicates the presence of C=C, O-H (alkenes, alcohol, Phenols) bond of strong intensity, spectrum shown in Appendix 3 to5.

Table 4.15 Effects of Sub-fraction 1 (Sub-F1) and Sub-fraction 2 (Sub-F2) of Fractions 6of *Tephrosia vogelii*on Serum Lipid Profile in Poloxamer 407 Induced Hyperlipidemic Rats

Groups (n=4)	Serum TC (mmol/l)	Serum TAG (mmol/l)	Serum HDL-c (mmol/l)	Serum LDL-c (mmol/l)
NC	1.93±0.38^{a}	0.47±0.21^{a}	0.57±0.21^{a}	1.17±0.51^{a}
HyperC	6.23±2.68^{b}	2.23±0.97^{b}	0.60±0.00^{a}	3.77±1.91^{b}
Hyper+F6 (20mg/Kg BW)	5.00±2.66^{ab}	1.17±0.67^{ab}	1.00±0.95^{ab}	3.00±2.05^{ab}
Hyper+Sub-F1(20mg/KgBW)	3.37±1.94^{ab}	0.90±0.95^{a}	1.47±0.61^{ab}	1.70±0.50^{ab}
Hyper+Sub-F2(20mg/KgBW)	3.53±0.81^{ab}	1.40±0.72^{ab}	1.20±0.17^{ab}	1.97±0.60^{ab}
Hyper+Std	2.00±0.10^{a}	0.50±0.17^{a}	1.67±0.49^{b}	1.20±0.00^{a}

Values are means ± SD of four determinations. Values with different superscripts in the same column are significantly different ($p<0.05$).

NC: Normal rats Control, HyperC: Hyperlipidemic rats Control, Hyper+Std: Hyperlipidemic rats + Standard Drug (Atorvastatin 10mg/kg), Hyper+Rf 0.75-TLC20: Hyperlipidemic rats + RF value 0.60 from TLC of Hexane /Ethylacetate 7:3 (20mg/kg), Hyper+F(F6)20: Hyperlipidemic rats + F(F6) from Column Chromato of Methanol /Water 5:5 (20mg/kg), Hyper+Rf 0.22-TLC20: Hyperlipidemic rats +Rf value 0.22 from TLC of Hexane /Ethylacetate 7:3 (20mg/kg),.

TC: Total cholesterol, TAG: Triacylglycerol, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol,

Table 4. 16a: Fourier Transform Infrared Spectroscopy of fraction 6 of aqueous leaves extract of *Tephrosia vogelii*

S/No	Absorption peak (cm^{-})	Intensity	Bond	Class of compound	Functional group
1	401.21	5.418 (s)	R-I	Alkyl halides	C-I
2	1049.31	40.387 (m-s)	Ar-O-R	Ethers	=C-O-C
3	1072.46	39.846(m-s)	R-CH2-OH, Ar-O-R	Alcohols, Ethers	O-H, =C-O-C
4	1118.75	40.464(m-s)	C=C-CRR'-OH	Alcohols, Phenols	O-H
5	1342.5	38.55(vs)	R-F	Alkyl halides	C-F
6	1419.66	32.078(s)	R-NO2	Nitro Compounds	N-O
7	1458.23	34.562(s)	RCH=CH2	Alkanes, Alkyls	C-H
8	1558.54	29.422(m-s)	R-C(O)-NH-R	Amides	N-H
9	1643.41	27.119(vw-m)	RCH=CH2,, R-C(O)-NH2	Alkenes, Amides	C=C, C=O
10	2114.05	47.916(m)	R-C≡C-H	Alkynes	C≡C

11	2931.9	34.863(s)	R-C(O)-OH	Carboxylic Acids, Phenols	O-H
12	3417.98	20.675 (s,w)	R-C(O)-OH, R-NH2	Carboxylic Acids, mines	C=O, N-H
13	3742.03	45.337(s)	R-C(O)-OH	Carboxylic Acids	C=O
14	3842.33	48.733(m-s)	R-C(O)-NH-R	Amides	N-H

Intensity abbreviations: vw = very weak, w = weak, m = medium, s = strong, vs = very strong

Table 4.16b: Fourier Transform Infrared Spectroscopy of Sub fraction 2 of fraction 6 of aqueous leaves extract of *Tephrosia vogelii*

S/No	Absorption peak (cm^{-})	Intensity	Bond	Class of compound	Functional group
1	447.5	2.7782(s)	R-I	Alkyl halides	C-I
2	609.53	2.7516(s)	R-Br	Alkyl halides	C-Br
3	671.25	2.7416(s)	R-C≡C-H	Alkynes	≡C-H
4	848.71	3.2986(s)	R-Cl	Alkyl halides	C-Cl
5	1149.61	2.8483(s)	R-O-R', RR'R"C-OH	Ethers, Alcohols	C-O
6	1411.94	2.9074()	R-NO2	Nitro Compounds	N-O
7	1519.96	2.6024(m-s)	R-C(O)-NH-R	Amides	N-H
8	1651.12	2.0941(s)	RCH=CH2, C=C-C(O)-OH	Alkenes, Carboxylic Acids	C=C, C=O
9	1743.71	2.7836(s)	R-CH=O	Aldehydes	C=O
10	1921.16	2.8865(s)	R-C(O)-Cl	Acyl Chlorides	C=O

11	2306.94	2.5779(m-s)	R-C≡N	Nitriles	C≡N
12	2376.38	2.4585(m-s)	R-C≡N	Nitriles	C≡N
13	2862.46	2.0586(m)	R-CH=O	Aldehydes	C=O
14	2931.9	1.8878(w-m)	RCH=CH2	Alkenes	=C-H
15	3410.26	0.4944(s-w)	C=C-CH2-OH, R-NH2	Alcohols, Amines	O-H, N-H
16	3749.74	1.405(w-s)	R-NH2, R-C(O)-OH	Amines, Carboxylic Acids	N-H, O-H
17	3865.48	1.4417(w-s)	R-NH2, R-C(O)-OH	Amines, Carboxylic Acids	N-H, O-H

Intensity abbreviations: vw = very weak, w = weak, m = medium, s = strong, vs = very strong

Table 4.16c: Fourier Transform Infrared Spectroscopy of Sub fraction 1 of fraction 6 of aqueous leaves extract of *Tephrosia vogelii*

S/No	Absorption peak (cm^{-})	Intensity	Bond	Class of compound	Functional group
1	447.5	3.0435(s)	R-Br	Alkyl halides	C-Br
2	609.53	2.9478(s)	R-Br, R-C≡C-R’	Alkyl halides, Alkynes	C-Br, ≡C-H
3	671.25	2.9294(s)	R-C≡C-H	Alkynes	≡C-H
4	848.71	2.7635(m-s)	RR’R”C-OH	Alcohols, Phenols	C-O, OH
5	1149.61	2.7433(m-s)	R-C(O)-NR’R”	Amides	N-H
6	1411.94	2.4693(s)	R-NO2	Nitro Compounds	N-O
7	1519.96	2.1647(w)	R-NH2	Amines	N-H
8	1651.12	2.6319(s)	Ar-C(O)-O-C(O)-Ar, R-C(O)-O-R	Anhydrides, Esters	C=O
9	1743.71	2.7205(s)	R-CH=O	Acyl Chlorides	C=O
10	1921.16	2.4866(s)	R-C(O)-Cl	Acyl Chlorides	C=O

11	2306.94	2.3828(m-s)	R-C≡N	Nitriles	C≡N
12	2376.38	2.056(s)	R-C(O)-OH, R-CH=O	Carboxylic Acids, Aldehydes	O-H, H-C=O
13	2862.46	1.9197(s)	R-C	Alkanes, Alkyls	C-H
14	2931.9	0.7476(s)	C=C-CH2-OH,	Alcohols, Phenols	O-H
15	3749.74	1.5126(w)	R-NH2, R-C(O)-OH	Amines, Carboxylic Acids	N-H, O-H
16	3865.48	1.5501(s)	R-NH2, R-C(O)-OH	Amines, Carboxylic Acids	N-H, O-H

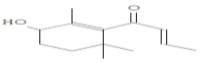
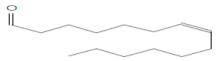
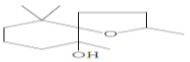
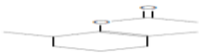
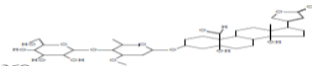
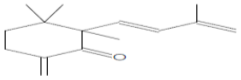
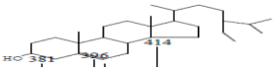
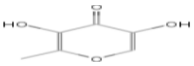
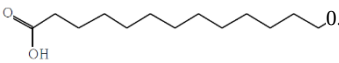
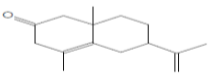
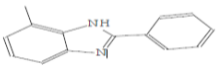
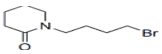
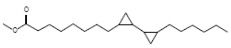
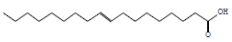
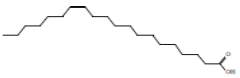
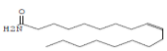
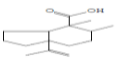
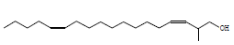
Intensity abbreviations: vw = very weak, w = weak, m = medium, s = strong, vs = very strong

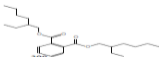
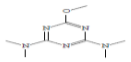
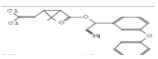
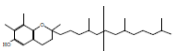
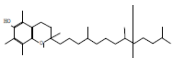
4.17Gas Chromatography-Mass Spectroscopy Analysis of bioactive from fraction 6 from Column Chromatography and two subfractions from TLC of *Tephrosia vogelii*

The tables of the bioactive from fraction 6 and two subfractions with Rf values 0.60 and 0.22 of *Tephrosia vogelii* revealed the presence of thirty (30), seventeen (17) and fifteen (15) peaks respectively. The active principles with their retention time (RT), compound names, molecular structures, concentration (peak area %) and molecular weight (MW) are presented in Table 4.17.1, Table 4.17.2 and Table 4.17.3 respectively. The GC-MS chromatograms of the thirty, seventeen and fifteen peaks of the compounds detected are shown in appendix 6, appendix 7 and appendix 8 respectively. The compounds identified by the mass spectroscopy are presented with compound name and percentage peak of the individual compounds. The results showed the presence of 7-Methyl-2-phenyl-1H-benzimidazole (33.47%),2-Piperidinone, N-[4-bromo-n-butyl]-(39.61%), gamma.-Tocopherol (1.33%), 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- (44.12%) and 2-Methyl-

Z,Z-3,13-octadecadienol (3.62%) as the compounds of antihyperlipidemic effects as shown in table 4.18.

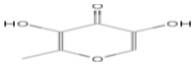
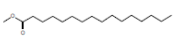
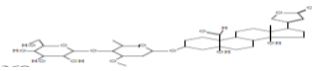
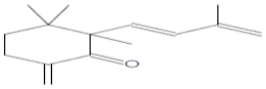
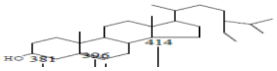
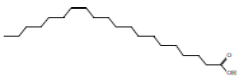
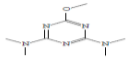
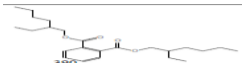
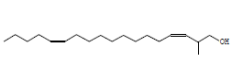
Table4.17.1: Major Compounds present in the bioactive fraction 6 of *Tephrosia vogelii*by GCMS analysis

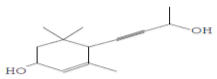
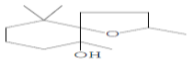
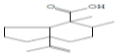
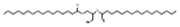
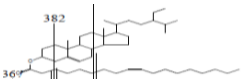
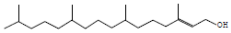
PN	RT	Compound Name	Structure	(Peak Area/SI)%	SI(%)	MW
1	9.169	:3-Hydroxy-.beta.-damascone		0.35	78	208
2	9.394	7-Tetradecenal,		13.71	86	210
3	9.926	2,6,10,10-Tetramethyl-1-xaspiro[4.5]decan-6-ol		1.46	76	212
4	10.073	1-Cyclohexen-1-ol,2,6-dimethyl-,acetate		1.27	73	168
5	10.403	3-[(2,6-dideoxy-4-O-.beta.-Dlucopyranosyl-3-O-methyl-.beta.-D-ribo-hexopyranosyl)oxy]-5		1.14	72	710
6	11.371	2,3,3-Trimethyl-2-(3-methylbuta-1,3-dienyl)-6-methylenecyclohexanone		0.29	72	218
7	11.608	.gamma.-Sitosterol		9.34	87	414
8	12.108	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-		44.12	94	140
9	12.880	Palmitic acid x2		0.57	95	270
10	13.083	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-		1.34	70	280
11	14.091	7-Methyl-2-phenyl-1H-benzimidazole		33.47	76	208
12	14.211	2-Piperidinone, N-[4-bromo-n-butyl]-		0.57	79	322
13	14.265	:[1,1'-Bicyclopropyl]-2-octanoic acid		0.47	79	322
14	14.615	9-Octadecenoic acid, (E)-		16.41	93	282
15	14.757	cis-13-Eicosenoic acid		2.25	75	310
16	15.950	9-Octadecenamide, (Z)-		3.47	85	281
17	16.332	7a-Isopropenyl-4,5-imethyloctahydroindene-4-carboxylic acid		1.16	74	236
18	16.774	2-Methyl-Z,Z-3,13-octadecadienol X2		3.62	90	280

19	16.983	Bis(2-ethylhexyl) phthalate		1.20	88	390
20	17.752	[1,3,5]Triazine-2,4-diamine, 6-methoxy-N,N,N',N'-tetramethyl-		5.71	70	197
21	18.584	Cypermethrin x3		3.57	95	415
22	20.501	gamma.-Tocopherol x3		1.17	84	416
23	21.412	Vitamin E		3.52	94	430

PN = peak number. SI = similarity index, MW = Molecular weigh

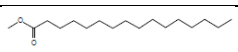
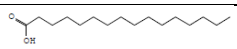
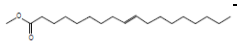
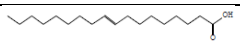
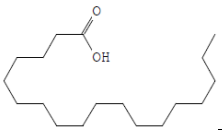
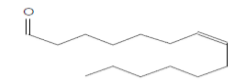
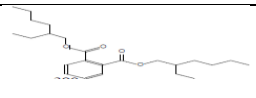
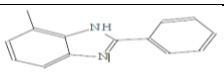
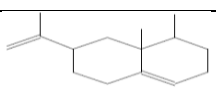
Table 4.17.2: Major Compounds present in the bioactive Sub fraction 2 of *Tephrosia vogelii*by GCMS analysis

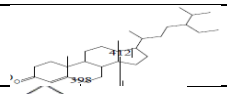
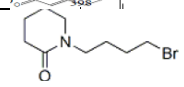
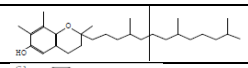
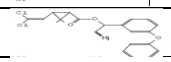
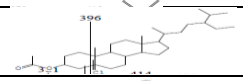
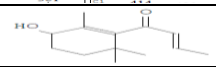
PN	RT	Compound Name	Structure	(Peak Area /SI) %	SI(%)	MW
1	12.009	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-		44.12	**94**	**140**
2	12.883	Palmitic acid, methyl ester x2		0.27	95	270
3	14.269	1-Cyclohexen-1-ol,2,6-dimethyl-,acetate		1.27	73	168
4	14.767	3-[(2,6-dideoxy-4-O-.beta.-Dlucopyranosyl-3-O-methyl-.beta.-D-ribo-hexopyranosyl)oxy]-5		1.14	72	710
5	15.573	2,3,3-Trimethyl-2-(3-methylbuta-1,3-dienyl)-6-methylenecyclohexanone		0.29	72	218
6	15.842	.gamma.-Sitosterol		9.34	87	414
7	16.777	cis-13-Eicosenoic acid		2.25	75	310
8	16.989	[1,3,5]Triazine-2,4-diamine, 6-methoxy-N,N,N',N'-tetramethyl-		5.71	70	197
9	17.522	Bis(2-ethylhexyl) phthalate		9.56	93	390
10	17.849	2-Methyl-Z,Z-3,13-octadecadienol		3.62	90	280

11	17.981	3-Hydroxy-7,8-dihydro-.beta.-ionol		0.23	72	208
12	18.795	2,6,10,10-Tetramethyl-1-xaspiro[4.5]decan-6-ol		1.46	76	212
13	19.279	7a-Isopropenyl-4,5-imethyloctahydroindene-4-carboxylic acid		1.16	74	236
14	20.513	Glycerol 1,2-dipalmitate		0.77	50	568
15	21.122	Stigmast-5-en-3-ol, oleate		0.91	80	678
16	21.901	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		0.57	84	296

PN = peak number
SI = similarity index
MW = Molecular weight

Table 4.17.3: Major Compounds present in the bioactive Sub fraction 1 of *Tephrosia vogelii*by GCMS analysis

PN	RT	Compound Name	Structure	(Peak Area/SI) %	SI(%)	MW
1	12.887	Palmitic acid, methyl ester		0.60	95	270
2	13.296	Palmitic acid		20.55	94	256
3	14.272	9-Octadecenoic acid, methyl ester, (E)-		1.62	93	296
4	14.628	9-Octadecenoic acid, (E)-		18.36	90	282
5	14.771	Octadecanoic acid		13.62	92	248
6	16.784	7-Tetradecenal,		13.71	86	210
7	16.993	Bis(2-ethylhexyl) phthalate		7.09	94	390
8	17.753	7-Methyl-2-phenyl-1H-benzimidazole		33.47	76	208
9	17.855	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-		10.89	80	204

10	18.768	4-Stigmasten-3-one		4.08	62	412
11	19.285	2-Piperidinone, N-[4-bromo-n-butyl]-		39.61	86	234
12	20.527	gamma.-Tocopherol		1.34	62	416
13	20.743	Cypermethrin		5.49	95	415
14	21.139	Stigmastan-3-ol, 5-chloro-, acetate,		3.40	67	492
15	21.794	:3-Hydroxy-.beta.-damascone		0.35	78	208

PN = peak number
SI = similarity index
MW = Molecular weight

Table 4.18: Activity of identified compounds

PN	Compound Name	MW	Compound nature	**Activity**	**References**
1	:[1,1'-Bicyclopropyl]-2-octanoic acid	322	Sesquiterpene compound,	Antimicrobial, Anti-inflammatory, Anti –hyperlipidemic	Jananie *et al*., 2011
2	9-Octadecenoic acid, methyl ester	296	Palmitic acid ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Anliandrogenic, Flavor, Hemolytic, 5 Alpha reductase inhibitor	Jegadeeswari *et al*., 2012
3	9-Octadecenoic acid, (E)-	282	Linoleic acid	HypocholesterolemicNematicide Antiarthritic Hepatoportective Anti androgeneic Hypocholesterolemic5-Alpha reductase Inhibitor Antihistaminic Anticoronary Insectifuge	Manonmani and Catharin, 2015
4	.gamma.-Sitosterol	414	Sterol	antimicrobial,anticancer antiinflammatory,antiasthma diuretic, hypocholesterolemic	Jegadeeswari *et al*., 2012
5	gamma.-Tocopherol	416	Vitamin	antioxidant activity antiageing, analgesic, antidiabatic,antiinflammatory, antioxidant,antidermatitic, antileukemic,antitumor, anticancer,hepatoprotective,hypocholesterolemic, antiulcerogenic, vahzodilator, antibronchitic,anticoronary	. Sathiyabalan *et al.*, 2014
6	Stigmastan-3-ol, 5-chloro-, acetate,	492	Sterol	Antimicrobial, Anticancer. Antiarthritic, Antiasthma, Diuretic, Anti-inflammatory and	Jegadeeswari *et al*., 2012

				Antihyperlipidemic	
7	2-Piperidinone, N-[4-bromo-n-butyl]-	234	Alkaloid	Antimicrobial, Antioxidant, Anti-inflammatory, Antihyperlipidemic	Meenakshi *et al*., 2012.
8	2-Methyl-Z,Z-3,13-octadecadienol	280	Alcoholic compound	Antimicrobial, Anti-inflammatory, Anti-Hyperlipidemic	Wright *et al*.,1996; Paul *et al*., 2012
9	7-Methyl-2-phenyl-1H-benzimidazole	208	Imine compound	Antimicrobial, Anticancer, Antiinflammatory, Antihyperlipidemic	Vildan *et al*., 2009
10	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	140	Flavonoid fraction	Antimicrobial, Anti-inflammatory, Antioxidant and Anti-hyperlipidemic	Jananie *et al*., 2011
11	7-Tetradecenal,	210	Aldehyde compound	Antimicrobial, Antiinflammatory, Antihyperlipidemic	Sujatha *et al*., 2014

CHAPTER FIVE

5.0 DISCUSSION

The inverse relationship between plants intake and the risk of oxidative stress associated diseases such as cardiovascular diseases has been partially ascribed to active components (Hollman, 1999). Therefore, it is reasonable to determine the active components content of extracts from different part of the plant. The present research was therefore designed to study the bioactivity guided fractionation of aqueous leaves extract of *T. vogelli* and its effect on hyperlipidemic rats.The presence of the secondary metabolites, alkaloids, saponins andflavonoidswhich have been reported to be responsible for protecting the plants (Smith and Eyzaguine, 2007; Kumar *et al*., 2009) and some associated with numerous physiological activities in mammalian cells in various studies (Mishra *et al*., 2009),may explain the various uses of *T. vogelii* for traditional medicine. The pharmacological and other beneficial effects of these secondary metabolites in plants have been reviewed by Soetan (2008).The quantitative analysis of phytochemical constituents of aqueous leaves extract of *T. vogelii* revealed

that the saponins is significantly ($p<0.05$) higher than the alkaloids and flavonoids. This may be responsible for the observed antihyperlipidemia as it has been reported to possesse hyperlipidemic activity (Murphy *et al.*,2007).

Aqueous extract had the highest yield compared to acetic acid and ethanolic extracts which could be due to the greater polarity of water this agrees with the work of Soetan (2008).

The Poloxamer 407 gave the best induction ofhyperlipidemia, via increased total cholesterol (TC), triacylglyceride (TAG) and low density lipoprotein (LDL) in hyperlipidemic rats(Table 3). It increases serum lipoproteins possibly via its actions at various levels in lipid metabolism, largely by inhibiting lipoprotein lipase, which facilitates the hydrolysis of triglycerides. P407 also causes indirect stimulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase which is involved in cholesterol biosynthesis (Hetal and Dion, 2013). The elevated lipid levels in the Poloxamer 407 and high fat diet induced hyperlipidemic rats indicate persistent hyperlipidemia in the rats used and corresponds well with results of previous works(Murphy *et al.*,2007; Nissen *et al.,* 2004). The six different extracts significantly($p<0.05$) reduced TC, TAG and LDL levels with aqueous leaves extract been the highest (Table 3). The highest percentage reduction found in the aqueous extract suggested that aqueous extract of the leaves of *T. vogelii*probablyinhibits more HMGCoA reductase activity.

It isvery necessary to determine LD_{50} in order to prevent eventualities of drug orcompounds overdose which may hinder the therapeutic value of the drug (Prohp and Onoagbe,2012). The study is also useful in understanding toxicity profiles of plant extracts (Ozbek*et al*., 2004). The result of acute oral toxicity study in the rats recorded

an LD_{50} of less than5000 mg/kg. For the aqueous leaves extract furthermore the rats tolerated the extracts without any symptoms of acute toxicity that is there was no mortality at first phase within 48 hours using lower doses; 10, 100, 1000, 1600 mg/kg body weight of aqueous leaf extract to albino rats even at larger doses of extracts administered. It has been reported that LowerLD_{50}implies a more toxic extract and may induce more animals. According to Lorke (1983), an LD_{50} valuebeyond 5000 mg/kg is of no toxicological significance.

Furthermore, after fractionation of the aqueous leaves extract, ten (10) fractions were obtained and subjected to antihyperlipidemic potential as well. From the results, fraction 6 showed significantly ($p<0.05$) higher attrnuation of dyslipidemia in the hyperlipidemic rats (Table 6). This shows that more of the active ingredients of *T, vogelii*are concentrated in the fraction 6 and thus, showed higher antihyperlipidemic action.

In this study aqueous leaves extract and fraction 6 of *T. vogelii* significantly ($p<0.05$) reduced TC, TG and LDL-c concentrations with fraction 6 showing the highest decrease. These reductions in TC, TG and LDL levels suggest the ameliorative potential of *T. vogelii* extract and fraction 6. It could also be due to the presence of saponins, a phytochemical which forms insoluble complexes with cholesterol or their bile salt precursor, thus making them unavailable for absorption (Messina, 1999).

Low density lipoprotein (LDL) is responsible for transporting cholesterol to the body cells. LDL is commonly known as the bad cholesterol, which is produced by the liver and carry cholesterol and other lipids from the liver to different areas of the body like muscles, tissues, organs and heart. The high levels of LDL indicate much more cholesterol in the blood stream than necessary and hence, increase the risk of heart

disease (Ahmed *etal*., 1998; Ginsberg and Goldberg, 2001; Costet, 2010). It was reported that some isoflavones (a type of flavonoid) increase resistance to LDL-c oxidation, like soybean isoflavones and genistein derivatives. Flavonoids acts as a cofactor of theenzyme cholesterol esterase, enhancing its activity (Lee *et al*., 1999). Flavonoids act on body lipid constituents like steroids and bile acids, and influence lipid metabolism. They increase bile acid excretion because cytochrome P- 450 enzymes bind some compounds to the bile acids and therefore reduce cholesterol level in the body (Di Carlo *et al*., 1992). Baum *et al.*,(1998)reported the triacylglycerol-lowering effect of flavonoids, while Kato and Tosa(1983) showed that quercetin added to the diet for 15 days reduced triacyglycerol levels in rats. A number of studies have shown that saponins when supplemented in diets reduce cholesterol content in blood and tissues in monogastric mammals, such as rats, gerbils and humans (Sidhu and Oakenfull, 1986; Potter *et al*., 1993; Harris *et al*., 1997). Alkaloids possess anti-hyperglycemic and anti-lipidemic effects on blood glucose and serum lipid profiles in streptozotocin-induced diabetic rats (Dineshkumar1 *et al*., 2010).Fraction 6 also shows significant ($p<0.05$) reduction in LDL-c levels which is in accordance with the work of Baum *et al.*, (1998), who reported that some active components may work by increasing LDL-c receptors densities in the liver binding to apolipoprotein B thereby making liver cells more efficient to remove LDL-C from blood.

HDL is commonly referred to as the good cholesterol, which is produced by the liver to carry cholesterol and other lipids from tissues back to the liver for degradation (Ginsberg and Goldberg, 2001; Ridke *etal*., 2010).High levels of HDL cholesterol have been considered as a good indicator of a healthy heart. The concentrations of 60 mg/dL or higher have been considered as optimal, whereas, HDL concentrations below 40 mg/dL are considered as major risk factor for CVDs. However, HDL is often interpreted in the context of

TC and LDL concentrations, and hence may be regarded as less significant when LDL is low. HDL-c acts as cholesterol scavengers, they pick up excess cholesterol and cholesterol esters from the blood and peripheral tissues to the liver where it is broken down to bile acids. It plays an important role in reducing blood and peripheral cholesterol concentrations and inhibits formation of atherosclerotic plaque in the aorta (Kim *et. al.*, 2008; Karmarkar, 2008) therefore known as the protective cholesterol. The present studies shows significant ($p<0.05$) increase in HDL-c by the leaves extract and fraction 6. This could possibly be due to increasing activity of lecithin-cholesterol acyl transferase (LCAT), an enzyme responsible for incorporating free cholesterol into HDL-c as suggested by Geetha *et al.*,(2011), there by promoting reverse cholesterol transport and competitively inhibiting the uptake of LDL-c by endothelial cells and preventing the generation of oxidized LDL-c (Yokozawa *et al.*, 2006).

Atherogenic indices are powerful indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease and the lower atherogenic index is protective against coronary heart disease (Ikewuch and Ikewuchi, 2010). The atherogenic index, a ratio of LDL-C to HDL-C is commonly used as an index to evaluate the risk for atherosclerosis as a result of increased HDL-C levels (Fki *et al.*, 2005). Therefore, elevating the level of HDL-C and lowering the atherogenic design are important measures in reducing the risk of atherosclerosis. Atherogenic indices are atherogenic coefficient, Cardiac risk ratio and Atherosclerosis index. Atherogenic risk predictor indices (HDL-c/TC, LDL-c/HDL-c and log (TG/HDL-c) are mathematical relationships between TC, TG, LDL-c and HDL-c that have been successfully used as markers of assessing atherosclerosis development (Nicholls *et al.*, 2007; Kastelein *et. al.*, 2008) and extent of CHDs. HDL-c/ TC ratio greater than 0.3 and LDL-c/HDL-c ratio less than 2.3 indicate a reduced risk of peripheral arterial disease (Ojiakor and Nwanjo, 2005). However, log (TG/HDL-c) has been considered the most

accurate in determining the extent of atherosclerosis and the risk of myocardial infarction (Dobiavosa *et. al.,* 2005). It has been suggested that log (TG/HDL-c) values of -0.3 to 0.1, 0.1 to 0.24 and above 0.24 are associated with low, medium and high cardiovascular risk disease respectively (Dobiasova, 2006). The study showed that the leaves extract and fraction 6 significantly ($p<0.05$) increased HDL-c/TC ratio, and lowered LDL-c/HDL-c and log (TG/HDL-c) ratio when compared with the induced not treated group. The results suggest the anti-atherogenic potential of *T. vogelii* leaves aqueous extract and fraction 6 and hence, reducing the development of coronary atherosclerosis as suggested (Dobiasova and Frohlich, 2001),F6 (200mg/kg) has ratio of HDL-c/TC reduction greater than 0.3 and the ratio of LDL-c/HDL-c less than 2.3.

Tissue damage is usually associated with the release of enzymes specific to the affected tissue or organ in circulation. The consequence is an increase in the activity of such enzymes in body fluids (Aliyu *etal*., 2006). Alanine aminotransferase (ALT) is a cytoplasmic enzyme found in very high concentration in the liver and an increase of this specific enzyme in the blood indicates hepatocellular damage, while aspartate aminotransferase (AST) is less specific than ALT as an indicator of liver function (Aliyu *etal.,* 2006). Aminotransferases are considered as indicators of hepatocellular health. Previous investigations indicated that high levels of these enzymes associated with later development of CVD and diabetes. AST, ALT and alkaline phosphatase (ALP) enzymes are released into the circulation from the cytosol and subcellular organelles of hepatocytes once liver is injured or damaged and their activities increased in blood (Ramaiah, 2007). There has been conflicting reports on the effect of poloxamer 407 induced hyperlipidemia on biochemical parameters related to hepatic functions (ALP, AST and ALT). Report on the effects of poloxamer 407 induced hyperlipidemia

on serum levels of the above enzymes showed that hyperlipidemia elevated serum levelsof ALT and AST (Hyeung *et al.,* 2006). Ameh *et al.,* 2013 found no effect on ALT except on AST, while Johnston *et al*., 1999 reported that P407 does not cause hepatic injury or damage. The discrepancy in the serum levels of the enzymes could be attributed to the levels and duration of hyperlipidemia (Lu *et al.,* 2007).

Hyperlipidemia is one of the disease conditions that are injurious to the liver; it sometimes results into fatty infiltration of the liver leading to a condition known as non-alcoholic fatty liver (Assy *et. al*., 2000). Fatty liver is an accumulation of triglycerides and other fats in the liver cells, if not treated leads to inflammation of the liver. It is characterized by varying degree of liver injury from steatosis to hepatitis, fibrosis and necrosis (Day and James, 1998). Cardiovascular disease is associated with high levels of circulatory cholesterol and other lipids (Huuponen *et al*., 1984) and this accounts for the atherosclerosis, arteriosclerosis and severe coronary heart disease which leads to increased levels of transaminases, marker enzymes important in heart and liver damage (Vaishwanar and Kowale, 1976). In this study, the elevated levels of AST, ALT and ALP observed in the serum of hyperlipidemic control may be due to injuries inflicted to the liver. This could be the accumulation of cholesterol, triglycerides and other fats in the liver cells and is in line with the work of Hyeung *et al.,* (2006). There was a significant ($p<0.05$) restoration of these liver marker enzyme levels in all the induced treated groups on administration of the extract, fraction 6 and atorvastatin. The significant($p<0.05$) reductions observed in the activity of ALT and AST indicate that the extract and fraction 6 of *T. vogelii* was able to ameliorate the liver damage. The reversal of this liver marker enzymes towards normalcy by the extract and fraction 6 observed in this study may be due to the prevention of the leakage of intracellular

enzymes by the presence of active components in the aqueous extract, fraction 6 and their membrane stabilizing activity (Muthu *et. al.,* 2008; Chavan *et. al*., 2012). This is in agreement with the previous study that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Chavan *et al*., 2012). It is therefore, a clear manifestation of hepatocurative effects of the aqueous leaves extract and fraction 6 which improve hepatic functions.

Serum levels of total bilirubin (TB) and direct bilirubin (DB) are some of the indices used to assess liver function as well as disease progression (Saad *et al.,* 2006; Uthandi and Ramasamy, 2011). The levels of these liver function parameters were affected as a result of fatty liver injuries inflicted by hyperlipidemia(Sheneni *et al*., 2014). Bilirubin is excreted by the liver and as such interference with the normal liver function which in turn affects its rate of conjugation or excretion. Thus a high level of bilirubin is used as indices for liver function and bile excretion status (Usha *et al.,* 2008). The present study showed a significant ($p<0.05$) decrease in the levels of direct bilirubin and increase in the levels of total bilirubin in hyperlipidemic control when compared to normal control. These changes could be as a result of fatty liver injuries inflicted by hyperlipidemia. Although, the levels are however restored towards normalcy by treatment with the plant extract and fraction 6. Thus, suggesting the enhancement of liver functions by the extracts and fraction 6.This is in line with the work of Sheneni *et al*., (2014) thatethanolic extracts of *Vitex donian* possesses hepatocuractive property in poloxamer induced hyperlipidemia.

Kidney helps in maintaining homeostasis of the body by reabsorbing important materials and excreting waste products (James *et al*., 2010).Its functionality is assessed

by determining theserum concentration of excretory constituents (Spencer *et al*., 2011). Creatinine is a waste product formed in muscle by creatine metabolism. Creatinine is the major catabolic product of the muscle and it is excreted in the kidneys. Creatinine levels are used as indicator of renal failure (Aliyu *et al*., 2006). Its retention in the blood is an evidence of kidney impairment. Urea is the main end product of protein catabolism. Amino acid deamination takes place in the liver where ammonia is converted into urea and excreted through urine. Urea varies directly with protein intake and inversely with the rate of excretion(Ranjna, 1999). Renal diseases which diminish the glomerular filtration lead tourea retention and decrease in urea is seen in severe liver disease with destruction of cellsleading to impairment of the urea cycle (Ranjna, 1999). Studies have shown that poloxamer (407) is non-toxic, safe for chronic administration (Megallis *et al.,* 2005) and capable of inducing hyperlipidemia in experimental animals (Johnston and Palmer,1997; Palmer*et al.,* 1998; Johnson *et al*., 2004).Hyperlipidemia is one of the disease conditions that are injurious to the kidney. It has been found to be capable of inducingglomerular injury (Tolins *et al*., 1992; Grone *et al*., 1994). The elevated levels of creatinine and urea observed in the serum of hyperlipidemic control compared with the group treated with fraction 6 is in line with the work of Khan and Siddique, (2012) that the accumulation of triglycerides and other fats in the kidney may be as a result of injuries or damage of the nephron structural integrity. However, there was a restoration of the kidney serum levels of creatinine and urea in all the induced treated groups on administration of the extract and fraction 6. This study agrees with the work of Aliyu et *al*., (2006) where ethanolic leaf extract of *Commiphora africana* (Burseraceae) was shown to decrease creatinine and urea in the serum blood. High blood urea is associated with increased tissue protein catabolism, excess break-down of blood protein and diminished excretion of urea.

An extensive range of antioxidant defenses, both the endogenous and exogenous are present to protect cellular components free radical-induced damage. These defenses include antioxidant enzymeslike superoxide dismutase (SOD) and catalase.Malondialdehyde (MDA) is a product of lipid peroxidation (Devaki *et al.,* 2004). The major product of lipid

peroxidationis MDA (Romero *et al.,* 1998).An increase in the serum MDA levels is an indication of elevated level of lipid peroxidation (Trible *et al.,* 1987). Therefore, measurement of MDA is an indirect method for assessing the extent of lipid peroxidation.*T. vogelii*extract and its fraction 6 treatment which caused a decrease in the levels of MDA is in accordance with the findings of Mathew *et al.,* (2007), which suggests that the seed of *Garcinia kola* may possess the natural antioxidants necessary for protectionagainst free radical damage induced by carbon tetrachloride in rat liver, since marked decrease in the levels of lipid peroxides was recorded in rats pretreated with *Garcinia kola* seeds.In this study, the liver and kidney antioxidant enzymes (SOD, CAT) activities significantly decreased in rats induced with hyperlipidemia compared to those in normal control group.The decrease in the activities of these enzymes could be attributed to the excessive utilization of these enzymes in inactivating the free radicals generated due to the induced hyperlipidemia (Ma *et. al.,* 2011). Our results are in agreement with Cui*et al*., (2011) who studied the effect of high fat diet in liver antioxidant enzyme systems. It has been also reported that oxidative stress increase SOD production (Mahfouz *et al*., 1997). Hyperlipidemia is associated with increased oxidative stress and overproduction of oxygen free radicals (Zalba *et al.*, 2001).

Oxidation is one of the destructive processes in which it breaks down and damages the various molecules. Oxidative stress is a causative factor that links hyperlipidemia with pathogenesis of atherosclerosis (Yang *et al.,* 2010). Hypercholesterolemia leads to increased production of oxygen free radical i.e. reactive oxygen species (ROS). These attacks induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA (Rajani and Ashok, 2009). ROS exerts its cytotoxic effect by causing oxidation of lipids i.e. lipid peroxidation which results in formation of MDA. The observed increase in lipid peroxidation (TBARS) in hyperlipidemic control rats is consistent with previous studies which have shown that poloxamer induced hyperlipidemia leads to increased lipid peroxidation (Dutta and Bishayi, 2009). Treatment with the extract and fraction 6 of *T. vogelii*significantly ($p<0.05$) reduced the TBARS concentration. This study indicates that *T. vogelii* had a free radical scavenging activity which probably provides organ protection from hypercholesterolemia. The ability of the extract and fraction 6 to inhibit the process of lipid peroxidation in hyperlipidemia ratsmay be due to the presence of saponins, flavonoids and alkaloids found in the preliminary phytochemical screening as previously been reported that saponins and flavonoids possess antihyperlipidemic activity(Ghule *et al.,* 2009; Patel *et al.,* 2009). According to previous reports a highly positive relationship between active compounds and antioxidant activity appears to be the trend in many plant species(Hideo *et al.,* 1993; Marja *et al.,* 1999; Ock *et al.,* 2003). This activity is believed to be mainly due to their redox properties (Zheng and Wang, 2001). The body also has an effective defence mechanism to prevent and neutralize free radicals-induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase. Under normal conditions, antioxidant enzymes such as superoxide dismutase catalyse the conversion

of superoxide radicals ($O^{2}-$) into hydrogen peroxide (H_2O_2) and O_2 (Oyedemi *et al*., 2010).Catalase further detoxifies H_2O_2 into H_2O and O_2 (Fridovich, 1986) while glutathione peroxidase also functions in detoxifying H_2O_2 like catalase. GSH plays an essential role in the liver by eliminating toxic compounds by conjugating them with GSH. However, imbalance between the formation of reactive oxygen species and their elimination occasioned by hypercholesterolemia has been implicated in oxidative-induced diseases.

The different effect of these extract and fraction 6 show that the extract and fraction 6 might have different effects on different antioxidant enzymes in liver and kidney, respectively, or the rat organs might absorb extract and fraction 6 differently and the metabolic enzymes in organs were different as well. Therefore, the activities of antioxidant enzymes may vary in different organs. We hypothesize that the change of enzyme activities is related to the components or metabolites of extract and fraction 6, which could affect enzymatic activities or enzyme contents. Further studies are needed to confirm this hypothesis. It can also be hypothesized that the anti-oxidant enzyme in liver and kidney may be up-regulated by administration of *T. vogelii* in response to hypercholesterolemia-enhanced free radical production.

Treatment with subfraction1 and 2 of F6significantly ($p<0.05$) reduced the TC.The results of total cholesterol, triacylglycerol and low density lipoprotein (LDL) showed a reduction in all treatment groups with subfraction1significantly ($p<0.05$) reduced the lipid levels. Study also showed that plasma HDL-cholesterol concentrationssignificantly ($p<0.05$) increased when compared to the hyperlipidemic contol. This is in agreement with the report of Malinon, (1997) who reported that the action of *Nauclea latifolia* in

reducing plasma TC, TAG and decreasing HDL concentrations could be due to the ability of one or more of the phytochemicals present in the plant to activate the functioning enzymes of the rat responsible for cholesterol absorption.

The Fourier transform infrared spectrometry, FTIR is a physico-chemical analytical technique that does not resolve the concentrations of individual metabolites but provides a profile of the metabolic composition of a tissue at a given time (Griffiths and de Haseth, 2007). Its spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of fraction 6 of *T. vogelii* leaves FTIR analysis showed the presence of alkyl halides, ethers, alcohol, nitro compounds, alkanes, carboxylic acids, phenol, amides, amines and alkynes compounds which showed major peaks at 401.21, 1049.31, 1072.46, 1419.66, 1458.23, 2931.9, 3842.33, 3417.98 and 2114.05 respectively. The prevailing functional groups that are found in sub fraction 2 FTIR analysis results were amines and alkynes compounds and that of sub fraction 1 were alcohol and phenol. The difference in functional groups between subfraction 1 and 2 may be responsible for their differences in antihyperlipidemic activities. This study agrees with the work of Lu*et al*.,(2004) who used extracts of spectrum of *Hypericum* and*Triadenum* with reference to their identification. Spectral differences are the objective reflection of componential differences by using FTIR spectrum, we can confirm the functional constituent's presence in the given parts and extract. We can also identify the medicinal materials from the adulterate and even evaluate the qualities of medicinal materials. The results of the present study spectrum also revealed the functional constituents present in the *T. vogelii*(Table 16). Many researchers applied the FTIR spectrum as a tool for distinguishing closely associated plants and other organisms (Hori and Sugiyama, 2003;

Lu *et al.*, 2004; Griffiths and de Haseth, 2007; Sahoo *et al*., 2011). O-H band (2931.9 cm-1) is a linkage absorption to part of gamma.-Tocopherol and 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-. Existence of –OH band in absorptions peak of FTIR spectrum was characteristic of gamma.-Tocopherol. Therefore, FTIR spectra represention indicate the sign of presence gamma.-Tocopherol and 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-like compounds in *T. vogelii*.

Gas Chromatography- Mass Spectrometry (GCMS) is a precious tool for reliable detection of bioactive constituents. The combination of a best separation technique (GC) with the best identification technique (MS) made GC/MS an ideal technique for qualitative analysis for volatile and semi-volatile bioactive compounds Grover and Patni, (2013). The GC/MS showed the relative concentration of various compounds getting eluted as a function of retention time.The GCMS results of subfraction 1,subfraction 2 and fraction 6of *T. vogelii* leaves analysis showed the presence of sixteen, fifteen andthirty compounds respectively(Table 17. 1, Table 17. 2 and Table 17. 3 respectively). This study agrees with the works of Keerthiga and Anand, (2015),Murugesan and Shanmugam, (2014) and Rajeswari and Muthuirulappan, (2015) who studieddifferent compounds fromdifferent plant species.

The results showed the pharmacological activities of the bioactive compounds(Table 18). Hexadecanoic acid is suggested to be a palmitic acid and it may act as an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic, 5 Alpha reductase inhibitor (Farina *et al*., 2014). 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- is a flavonoid fraction and it acts as an antihyperlipidemic, antimicrobial, anti-inflammatory and antioxidant (Jananie *et al*., 2011). Octadeconoic acid is suggested to be an oleic acid in nature and acts as an anti-inflammatory,

anticancer, hypocholesterolemic, 5-Alpha reductase inhibitor and antiandrogenic activity (Manonmani and Catharin, 2015). 2-Piperidinone, N-[4-bromo-n-butyl] - is recommended to be an alkaloid and it employed as an antimicrobial, antioxidant, anti-inflammatory and antihyperlipidemic agent (Meenakshi *et al*., 2012). 2-Methyl-Z,Z-3,13-octadecadienol is an alcoholic compound and acts as an antimicrobial, anti-inflammatory, anti-hyperlipidemic agent (Wright *et al*.,1996; Paul *et al*., 2012). 7-Methyl-2-phenyl-1H-benzimidazole is suggested to be an imino compound and it may act as an antimicrobial, anticancer, antiinflammatory and antihyperlipidemic agent (Vildan *et al*., 2009). 7-Tetradecenal is suggested to be an aldehyde compound and it may acts as an antimicrobial, anti-inflammatory and antihyperlipidemic agent (Sujatha *et al*., 2014). Gamma.-Sitosterol is a steroid and it acts as an antimicrobial, anticancer anti-inflammatory, antiasthma diuretic and hypocholesterolemic (Jegadeeswari *et al*., 2012). Stigmastan-3-ol, 5-chloro-, acetate is a steroid and it acts as an antimicrobial, anticancer, ant arthritic, antiasthma, diuretic, anti-inflammatory and antihyperlipidemic (Jegadeeswari *et al*., 2012). Gamma.-Tocopherol is a vitamin compound and it acts as an antioxidant activity, antiageing, analgesic, antidiabatic, antiinflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic and anticoronary (Sathiyabalan *et al.*, 2014). Hence this high hypolipidemic activity of the fraction 6 and subfractions 1 and 2 were attributed to the presence of gamma tocopherol, 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-, octadeconoic acid, 1, E-11,Z-13-octadecatriene, 2-Piperidinone, N [4-bromo-n-butyl]-,2-Methyl-Z,Z-3,13-octadecadienol,7-Methyl-2-phenyl-1H-benzimidazole and 7-Tetradecenal. The ortho- and para- hydroxylated metabolites of gamma tocopherol and 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- are responsible for 70% of systemic inhibition of HMG-CoA

reductase activity (Jacobsen *et al*., 2000). Phenols possess direct or indirect hypocholesterolemic activity (Nash and Nash, 2008). Gamma tocopherol inhibits HMG-CoA reductase activity (Sujatha *et al*., 2014; Norazlina *et al*.,2007; Rashid *et al*., 2008), inhibits expression of cell adhesion molecules (Ahmad *et al*., 2005), reduces the levels of blood cholesterol (Shibata *etal*., 2008; Wu *et al*., 2008) and inhibition of lipid peroxidation (Jiang , 2009; Comitato *et al*., 2009). The o- and p-OH groups have the best electron-donating properties and confer higher stability to the radical form and reacting with HMG COA reductase active site preventing HMG COA (Jacobsen *et al*., 2000).*T. vogelii* is potent against hyperlipidemia and may have potentials for use in the preparation of antihyperlipidemic drugs.

CHAPTER 6

6.0 SUMMARY, CONCLUSION AND RECOMMENATIONS

6.1 Summary

The major findings of this investigation can be summarized as follows:

- The phytochemical screening of powdered sample of leaves and stem of *T. vogelii* revealed the presence of alkaloids, saponins, phlobatannins and flavonoids in powdered sample of leaves and stem of *T. vogelii.*
- The percentage yield of the leaves and stem of aqueous, acetic acid and ethanolic, extracts of *T. vogelii*(Table2) showedthat aqueous leaves extract is 18.21$\pm$1.12, ethanolic leaves extract is 12.24 $\pm$ 3.25 and stem acetic acid extract is 7.11$\pm$ 1.20.

- Preliminary antihyperlipidemic effect of the aqueous, ethanol, and acetic acid extracts of *T. vogelii* leaves and stem on poloxamer 407 and high fat diet induced hyperlipidemic rats *in vivo* revealed that Poloxamer 407 significantly ($p<0.05$) increase the lipid profile than the high fat diet and aqueous leaves extract significantly ($p<0.05$) lowered the TC and TAG
- The quantitative analysis of phytochemical constituents of aqueous leaves extract of *T. vogelii* revealed that the saponins is significantly ($p<0.05$) higher (9.00 ± 0.12) followed by flavonoids (0.62 ± 0.02) and the least Phenols (0.04 ± 0.01).
- Antiyperlipidemic effect of ten fractions (F1-F10) of *T. vogelii* revealed that fraction 6 (F6) significantly ($p<0.05$) lowered the TC and TAG.
- Antihyperlipidemic effect of twosubfractions of fraction 6 of *T. vogelii* revealed that subfraction1 significantly ($p<0.05$) lowered the TAG.

- The FTIR of subfraction1 showed the presence of phenols, alcohols, alkanes, alkyl halides, carboxylic acids, aromatics and nitro compounds. The prevailing functional groups were phenols andalcohols.
- The GC-MS analysis of subfraction 1 showed the presence of fourteen compounds. The prevailing compounds were gamma-tocopherol and other compounds that have antihyperlipidemic effect including 4H-pyran-4-one,3,5-dihydroxy, 2-methyl-Z,Z-3,13-octadecadienol and 9-Octadecenoic acid, (E)-.

6.2 Conclusion

The study demonstrated that aqueous leaves extract of *T. vogelii*possess antihyperlipdemic and antioxidant activities especially the fraction 6 was found to be more active. The isolation and partial purification of the two subfractions (1 and 2) revealed to be more potent. The FTIR of subfraction 1 revealed the presence of alcohol and phenol while GCMS revealed the presence of gamma.-Tocopherol compound; other compounds of anti-hyperlipidemic effect are 4H- Pyran-4-one, 3,5-dihydroxy-2-methyl- , 2-Methyl-Z,Z-3,13-octadecadienol and 9-Octadecenoic acid, (E)-.

6.3 Recommendation

(i)From these studies, we recommend that the extracts (acetic acid, ethanol and aqueous) of *T. vogelii* possess anti-hyperlipidemic potentials and thus its inclusion in traditional medicine as herbal medicine may be justified but after intensive toxicological evaluation.

(ii) Further work should be done on subfraction 1 of fraction 6 in the area of bioassay and structural elucidation using the combination of NMR, infrared spectroscopy and mass spectroscopy.

(iii) The single or multi-bioactive compounds isolated should be subjected to acute and chronic toxicological evaluation in order to determine their safe use and doses.

REFERENCES

Abourbih, S., Filion, K.B. and Joseph, L. (2009). Effect of fibrates on lipid profiles and cardiovascular outcomes: a systematic review. *American Journal Medicine*, 122 (10):962-968.

Adaudi, A.O., Aluwong, T., Salawu, O.A. and Anuka, J. A. (2009). Blood pressure, analgesic
and anti-inflammatory properties of methanolic extracts of *Tephrosia vogelii* in experimental animals. *Nigerian Veterinary Journal*, 30: 37-43.

Adebayo, A. H., Aliyu, R., Gatsing, D. and Garba, L. H. (2006). The effect of ethanolic leaf
extract on *Commiphora africana* (buseraceac) on lipid proile in rats. *International Journal of Pharmacology,* 2(6):618-622.

Adewunmi, C.O. and Ojewole, J.A.O. (2004). Safety of traditional medicines, complementary and alternative medicines in Africa. *African Journal of Traditional Complementary and Alternative Medicine*, 1: 1-3.

Adeyemi, O.O., Okpo, S.O. and Ogunti, O.O. (2002). Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea Americana Mill* (Lauraceae) *Fitoterapia*; 73:375-380.

Agbon,A., Ofojekwu, C. and Ezenwaka, I. (2004). Acute toxicity of water extract of *Tephrosia vogelii* Hook to species relevant in aquaculture ponds: rotifers, Cyclops, mosquito larvae and fish. *Journal of Applied. Ichthyology*, 20, 521–524.

Achinewu, J.O., Adesokan, A.A., Olatunji, L.A., Buoro, D.O. and Soladoye, A.O. (1995). Effect of Ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats. *Biochem*istry, 17(3): 45-47.

Adewoye, S. O. (2010). Haematological and biochemical changes in *Clarias gariepinus* exposed to *Trephosia vogelii* extract. *Advances in Applied Science Research*, 1 (1): 74-79.

Edijala, J.K., Asagba, S.O., Eriyamremu, G.E. and Atomatofa, U. (2005). Comparative effects of garden egg fruit, Oat and Apple on serum lipid profile in rats fed a high cholesterol diet. *Pakistan Journal of Nutrition*, 4: 245-249.

Ahima, R.S. (2006). Obesity epidemic in need of answers. *Gastroenterol,*131(4): 991-997.

Ahmad, N.S., Khalid, B.A., Luke, D.A. and Ima Nirwana, S. (2005). Tocotrienol offers betterprotection than tocopherol from free radical-induced damage of ratbone. *Clinical Experiment Pharmacology Physiology,* 32(9):761–770.

Ahmed, S.M., Clasen, M.D. and Donnelly, M.D. (1998). Management of dyslipidemia in adults. *American Family Physician*, 57(2): 1-16.

Akpa, L. E., AJIMA, M., Nwigwe, O., AUDU, B. S. and LABTE, S. M. (2010). Effects of
fish bean (*Tephrosia vogelii*) leave extract exposed to freshwater cichlid fish – *Tilapia zilli.Animal Research International,* 7(3): 1236 – 1241.

Akthar, R., Ahmad, S., Khan, M., Shaikh, T. and Mujawar, T. (2011). Antihyperlipidemic
effect of ethanolic extract of leaves of *Tephrosia purpurea* Linn in dexamethasone induced rats. [Article and details unavailable]

Alao, F. O., Adebayo, T. A. (2015). Comparative efficacy of *Tephrosia vogelii* and *Moringa*
*oleifera*against insect pests of watermelon (*Citrullus lanatus* Thumb). *International Letters of Natural Sciences Online. 35, 71-78.*

Aliyu, R., Adebayo, A.H., Gatsing, D. and Garba, I.H. (2006). The Effects of Ethanolic Leaf Extract of *Commiphora Africana* (Burseraceae) on Rat Liver and Kidney Functions. *Journal of Pharmacological Toxicology,* 2(1): 373-379.

Ameh, D.A., Adejo, E.B. and James, D.B. (2013). Effect of *Garcinal kola* biflavonoid fractions on some biochemical parameters of P407-induced hyperlipidemia rats: A phytopreventive and phytotheraupeutic studies. *Journal of Comparative Clinical Pathology*, 2(3): 23-25.

American Heart Association. Scientific statement on Cholesterol and Hyperlipidemia. 2005: 1-2.

American Heart Association: Heart Disease and Stroke Statistics. (2008). Update. AHA. Texas.

Angela, M. G. (2008). High Fat Diets for Diet-Induced Obesity Models A Brief Review of the Scientific Literature Obesity-3000-2-09.

Anup, K. M., Smriti, T., Zabeer, A. and Ram, K. S. (2012). Antidiabetic and
antihyperlipidemic effect of *Euphorbia hirta* in streptozotocin induced diabetic rats. *Der Pharmacia Lettre,* (2):703-707.

Assy, N., Kaita, K., Mymin, D., Levy, C., Rosser, B. and Minuk, G. (2000). Fatty infiltration of liver in hyperlipidemic patients. *Digestive Diseases and Sciences, 45*(10): 1929-1934.

Baron, R.B. (2005). Lipid Abnormalities. In: Current Medical Diagnosis and Treatment. The McGraw-Hill Company, 44th ed.: 1202.

Bartels H. and Bohmer M. (1973). A kinetic method for measurement of serum creatinine. *Medicine Laboratory,* 26(9): 209-215.

Baum, J.A., Teng, H., Erdman, J.W., Weigel, R.M., Klein, B.P., Persky, V.W., Freels, S., Surya, P., Bakhit, R.M., Ramos, E., Shay, N.F. and Potter, S.M. (1998). *American Journal of Clinical Nutrition,* 58: 545.

Bell, D.S.H. and O'Keefe, J.H. (2009). Rediscovering bile acid sequestrants. *Diabetes Obese Metabolism,* 11 (12):11-14.

Belhekar, P. D., Chaudhari1,S. N., Saryawanshi,J. S., Mali, K. K. And. Pandhare, R. B. (2013). Antidiabetic and antihyperlipidemic effects of *Thespesia populnea* fruit pulp
extracts onalloxan-induced diabetic rats. *Indian Journal of Pharmaceutical Sciences*, 13 (1): 1345-138.

Bennett, D.R. (1995). Drug Evaluation Annual. Published by the American Medical Association: 2455-2500.

Bhatnagar, D., Soran, H. and Durrington, P.N. (2008) Hypercholesterolaemia and its management. *Biology Medicine Journal*,4 (2) :337- 993.

Bibitha, B., Jisha, V. K., Salitha, C.V., Moha, S. and Valsa, A.K. (2002). Indian *Journal of Microbiology*. 42: 361 -363.

Boham, B.A. and Kocipal-Abyazan, R. (1974). Flavonoids and condensed tannins from leaves of Haiwaiian *Vaccinium vaticulatum* and *Vaccinium calycinium*. *Asian Journal of Pacific Science*, 48(5): 458-463.

Braamskamp, M.J., Wijburg, F.A. and Wiegman, A. (2012). Drug therapy of hypercholesterolaemia in children and adolescents. *Drugs*, 72 (7): 759-762.

Braiet, B.I., Odetola, A.A. and Agomo, P.U. (2007). Hypoglycemic and hypocholesterolemic potential of *Persea americana*leave extracts. *Journal of Medicine and Food*, 10:356-360.

Breazile, H. (1971). Effects of drying and storage of herbs and spices on the essential oil of *O. basilicum*. *Flavour Fragrance Journal,* 7(9): 267-271.

Brian, F.H., Thomas-Bigger, J. and Goodman, G. (1985). The pharmacological basis of therapeutics, seven Edition, Macmillan Publishing Company, New York, pp 716-718.

Brown, S.L. (1996). Lowered serum cholesterol and low mood. *Britain Medicine Journal*, 3(13): 637-638.

Buragohain, J., Konwar, B. K. and Bordoloi, M.J. (2012). Der Pharmacia Sinica, (6), 149-152.

Buringh, E. and Opperhuizen, A. (2002). RIVM rapport 650010032, on health risks of ambient PM in the Netherlands, Bilthoven, and The Netherlands

Burkill, H. (1985). The useful plants of west tropical Africa, Entry for *Peristrophe bicalyculata* Endocrinology, *Metabolism Clinics of North America*, 19 (2): 259-278.

Castilla-Guerra, L., Fernández-Moreno, M. C and Alvarez-Suero, J. (2009). Secondary stroke prevention in the elderly: new evidence in hypertension and hyperlipidemia. *European Journal International Medicine*, 20(5): 586 -589.

Chait, A.and Brunzell, J. D. (1990).”Acquired hyperlipidemia (secondary dyslipidemia)” of *Butea monosperma* (Lam) Leaves on Paracetamol Induced Hepatotoxicity in Albino Rats. *Journal of Pharmacy Research*, *5*(8):4006-4008.

Challa, V. (1999). Atherosclerosis of the cervicocranial arteries. In Toole JF (ed) Cerebrovascular disorders. 5th edition. Lippincott Williams and Wilkins, Philadelphia.

Chavan, S.D., Patil, S. B. and Naikwade, N. S. (2012). Biochemical and Histopathological Studies (Retz) Nees [family ACANTHACEAE]. Vol. Royal Botanical Gardens. http://www.aluka.org, September 5,

Cheeke, P.R. (1989). Toxicants of Plant origin. CRC Press, Boca Raton, Florida, Pp 37-39.

Chopra, R. N.; Naygar, S.L. and Chopra, K. (1956). Glossary of Indian Medicinal Plants. Council of Sci. Industrial Res. New Delhi, p 160.

Choudhary, M. I., Naheed, S., Jalil, S., Alam, J. M. and Atta-Ur, r. (2005). Effects of ethanolic extract of (*Iris germanica)* on lipid profile of rats fed on a high fat diet. *Journal of Ethnopharmacology*, 98: 217-220.

Comitato, R., Nesaretnam, K., Leoni, G., Ambra, R., Canali, R., Bolli, A., Marino, M. and Virgili, F. (2009). A novel mechanism of natural vitamin E tocotrienol activity: involvement of ERbeta signal transduction. *American Journal of Physiology Endocrinology Metabolism*, 297(2):427–437.

Corsini, A., Windler, E. and Farnier, M. (2009). Colesevalam hydrochloride: usefulness of a specifically engineered bile acid sequestrant for lowering LDLcholesterol. *European J0urnal of Cardiovascular Prevention and Rehabilitation*, 16(1):1-9.

Costet, P. (2010). Molecular pathways and agents for lowering LDL-cholesterol in addition to statins. *Pharmacology Therapy*, 126(3): 263-781.

Cui B.K., Liu S., Lin X.J., Wang J., Li SH., Wang Q.B. and Li S.P. (2011). Effects of *Lycium barbarum* aqueous and ethanol extracts on high fat-diet induced oxidative stress in rat liver tissue. *Molecules,* 16 (8):9116–9128.

Curl, C.L., Price, K.R. and Fenwick, G.R. (1988). The quantitative estimation of saponins in Pea (Pisum sativum) and Soya (Glycine max). *Food Chemistry*, 18(6): 241- 250.

Dai, S. and McNeill, J.H. (1995). Fructose induced hypertension is concentration and duration dependent. *Journal of Pharmacology and Toxicology Methods*, 33:101-107.

Daisy, P., Kanakappan, S. and Rajathi, M. (2009). Antihyperglycemic and antihyperlipidemic effects of *Clitoria ternatea* Linn. in alloxan-induced diabetic rats. *African Journal of Microbiology Research*, 3 (5): 27-29.

Dargel, R. (1989). Lipoproteins and the etiopathogenesis of atherosclerosis. Zentralbl Allg Pathol.; 135: 501.

Das, K., Tiwari, R. K.S. and Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*, 4(2): 104-111.

Davidson, M.H. (2001). Safety profiles for the HMG-CoA Reductase Inhibitors. *Drugs*, 61(2): 197-206.

Day, C.P. and James O.F. (1998). Steatohepatitis: *Gastroenterology*, *114*(4): 842-845.

De, S., Dey, Y.N. and Ghosh, A.K. (2010). Phytochemical investigation and hromatographic
evaluation of the different extracts of tuber of *amorphaphallus paeoniifolius* (araceae), *International Journal of Pharmacology Biomedicine Reserve,* 1(5): 150-157.

DelgadoRoche, L., FragaPerez, A., BequerViart, M. A.and Hernandez-Matos, Y. (2012).
Lipofundin 20% induces hyperlipidemia and oxidative stress in male Sprague Dawley rats. *Veterinary World*, 5 (3):133-137.

Delvin, T.M. (2006). Normal Clinical Values: Blood. Textbook of Biochemistry with clinical correlation, six Edition.

Devaki, T., Raghavendran, H.R.B. and Sathivel, A. (2004). Hepathoprotective nature of seaweed alcoholic extract on acetaminophen-induced hepatic oxidative stress. *Journal of Health Science*, 50: 42-46.

Di Carlo, G., Autore, G., Izzo, A.A., Miolino, P., Diurno M.V. and Passo, F. (1992). *Journal*
of Pharmacology,45(8):4-9.

Dineshkumar, B., Analava, M. and Manjunatha, M. (2010). Antidiabetic and hypolipidemic
effects of mahanimbine (carbazole alkaloid) from *murraya koenigii* (rutaceae) leaves. *International Journal of Phytomedicine,* 2(11): 22-30.

Diodato, M. and Chedrawy, E. G. (2014). Coronary artery bypass graft: the past, present, and
future of myocardial revascularization. *Surgery Research and Practice,* Retrieved from http://dx.doi.org/10.1155/726158

Dobiasova, M. (2006). Atherogenic Index of plasma as a significant predictor of cardiovascular risk from research to practice. *VnitrniLekarstvi,* 52(1): 64 71.

Dobiasova, M. and Frohlich, J. (2001). The plasma parameter Log (TAG/HDL-c) as an atherogenic index: correlation with lipoprotein particle size and etherification rate in apoB-lipoprotein-depleted plasma (FERHDL). *Journal of Clinical Biochemistry,* 12(3): 588-592.

Dobiavosa, M., Urbanovan, Z. and Samanek, M. (2005). Relation between particle size of HDL and LDL Lipoproteins and cholesterol Esterification rate. *Physiological Research,* 54(4):159-165.

Doumas, B.T., Perry, B.W., Sasse, E.A. and Straumfjord, J.V. (1973). Standardization in bilirubin assays: Evaluation of selected methods and stability of bilirubin solutions.

Duff, R. C. (2008). *Test of Hypothesis in Population Means. In: Introductory Biostatistics for the Health Sciences*. John Wiley and Sons Inc. NY pp. 71-96.

Dutta, K. and Bishsayi, B. (2009**).** *Escherichia coli* lipopolysaccharide administration alters antioxidant profile during hypercholesterolemia. *Indian Journal of Clinical Biochemistry*, *24*:179–183.

Dzenda, T., Ayo, J.O., Adelaiye, A.B., Adaudi, A.O. and Ibrahim, N.D. (2008). Preliminary
investigation intothe acute oral toxicity of *Tephrosia vogelii* leaves in mice. *Nigerian Veterinary Journal* 28,47-52.

Ellis, D.I., Broadhurst, D., Kell, D.B., Rowland, J. J. and Goodacre, R. (2002). Rapid and
antitativedetection of the microbial spoilage of meat by Fourier transform infrared spectroscopy and machine learning. *Applied and Environmental Microbiology*; 68:2822-2828.

Ellman, B.J. (1959). Hypertriglyceridemia increases risk of cardiovascular diseases. *Journal of Medical Research, 108*(7): 77-80.

Estadella, D., Oyama, L., M., Damaso, A. R., Ribeiro, E. B. and Oller -Ascimento, C. M.
(2004). Effect of palatable hyperlipidic diet on lipid metabolism of sedentary and exercised rats. *Nutrition,* 20(2): 218-224.

Evans, W.C. and Trease, G.E. (2002). *Trease and Evans Pharmacognosy*. W.B. Saunders, China, pp. 193-407

Ezetimibe, (2004). A new cholesterol-lowering drug. *Drug Therapy Bull*. ; 42 (9):65-67.

Fajimi, A. K. and Taiwo, A. A. (2005). Herbal remedies in animal parasitic diseases in Nigeria: A Review*African Journal of Biotechnology*, 4 (4):303 -307.

Farina, M., Preeti, B. and Neelam, P. (2014). Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from Leaves of *Aegle marmelos. Biology Medicine Research International*, 6(1): 1-11

Fawcett, J.K. and Scott, J. (1960). A rapid and precise method for the determination of urea. *Journal of Clinical Pathology*, *13*(2): 156-159.

Fazio, S., Guyton, J.R., Polis, A.B., Adewale, A.J., Tomassini, J.E., Ryan, N.W. and Tershakovec, A.M. (2010). Long-term safety and efficacy of triple combination ezetimibe/simvastatin plus extended-release niacin in patients with hyperlipidemia. *America Journal of Cardiology*, 105 (6): 487-941.

Fki, I. M., Bouaziz, Z. and Sahnoun, S. (2005). Hypocholesterolemic effects of phenolic-rich extracts of *Chemlali olive* cultivar in rats fed a cholesterol-rich diet. *Bioorganic Medicine. Chemical*, 13 (3): 5362-5370.

Fraga, C.G., Leibovitz, B. E. and Toppel, A.L. (1988). Lipid peroxidation measured as TBARS in tissue slices: Characterization and comparison with homogenates and microsomes. *Free Radical Biology and Medicine*, 4(6): 155-161.

Fridovich, I. (1986). Oxygen radicals from acetaldehyde. *Free Radical in Biology and Medicine*, 7: 557-558.

Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma without Use of the preparative ultracentrifuge. *Clinical Chemistry*, 18, 499–502.

Fryar, C.D., Hirsch, R., Eberhardt, M.S., Yoon, S.S. and Wright, J.D. (2010). Hypertension, High Serum Total Cholesterol, and Diabetes: racial and ethnic prevalence differences in U.S. adults, NCHS Data Brief pp 36: 1-8.

Fuller, J. H., Shipley, M. J., Rose, G., Jarrett, R. J. and Keen, H. (1980). Coronary-heart disease risk and impaired glucose tolerance. *The White Study. Lancet*. 1:1373-1376.

Gadzirayi, C., Mutandwa, E., Mwale, M. and Chindundu, T. (2010). Utilization of *Tephrosia*
*vogelii*in controllingticks in dairy cows by small- scale commercial farmers in Zimbabwe. *African Journalof Biotechnology*,8, 4134-4136.

Ganesh, V., Halade, 1. and Archana, R. J. (2006). Effect of S*pirulina platensis* pretreatment
on isoproterenol induced hyperlipidemia in rats. *Pharmacologyonline* 2: 243-251.

Garcia, J.H., Ho,K. L., Pantoni, L., Henry, J.M., Mohr, J.P., Stein, B.M. and Yatsu, F.M.
(1998). Stroke Pathophysiology, Diagnosis andManagement. Third edition,
Philadelphia, PA: Churchill Livingston.

Gazi, I.F., Daskalopoulou, S.S. and Nair, D.R. (2007). Effect of ezetimibe in patients who cannot tolerate statins or cannot get to the low density lipoprotein cholesterol target despite taking a statin. *Current Medicine Research Opin*, 23(9):2183-2192.

Geetha, G., Kalavalarasariel, G.P. and Sankar, V. (2011). Antidiabetic effect of *Achyranthesru brofusca leaf* extracts on alloxan induced diabetic rats. *Pakistan Journal of Pharmaceutical Science*, 24(2): 193-199.

Ghule, B.V., Ghante, M.H., Saoji, A.N. and Yeol, P.G. (2009). Antihyperlipidemic effect of the methanolic extract from *Lagenaria siceraria* Stand fruit in hyperlipidemic rats. *Journal of Ethnopharmacology*, 124:333–337.

Ginsberg, H.N. and Goldberg, I.J. (2001). Disorders of Intermediary Metabolism (Disorders of lipoprotein metabolism). In: Principles of Internal Medicine fifteen edition.

Gomes, S.M. (1998). Efeitos de flavonoides no metqabolismo lipídico. Viçosa.
UniversidadeFederal de Viçosa. Dissertação de Mestrado.

Gramza, A. and Korozak, J. (2005). Trends Food Sci. Tech., 16: 351. Jackson, R., Chambles, L., Higgins, M., Kuulasmaa, K., Wijnberg, L. and Williams, D. (1999). (WHO MONICA project, and ARIC Study). Sex difference in ischaemic heart disease mortality and risk factors in 46 communities: an ecologic analysis. *Cardiovascular Risk Factors*, 7: 43-54.

Griffiths, P. and Haseth, J. A. D. (2007). Fourier Transform Infrared Spectrometry, John Wiley and Sons, In, second edition.

Gröne, E.F., Walli, A.K., Gröne, H.J., Miller, B. and Seidel, D. (1994). The role of lipids in nephrosclerosis and glomerulosclerosis. *Atherosclerosis*, *107*(1): 1-13.

Grover, N. and Patni, V. (2013). Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of *Woodfordia fruticosa* (L) Kurz. Leaves. *International Journal of Pharmacy and Pharmacology Science*, 5(4):291-295.

Grundy, S.M., Balady, G.J. and Criqui, M.H. (1998). Primary prevention of coronary heart disease: guidance from Framingham: a statement for healthcare professionals from the AHA Task Force on Risk Reduction. American Heart Association. *Circulation*, 97(18), 1876.

Guyton, J.R., Brown, B.G., Fazio, S., Polis, A., Tomassini, J.E. and Tershakovec, A.M. (2008). Lipid-altering efficacy and safety of ezetimibe/simvastatin coadministered with extended-release niacin in patients with type IIa or type IIb hyperlipidemia. *Journal of American Collaborate Cardiology*, 51(16):1564.

Guzik, T.J., Musa, S., Gastaldi, D., Sadowski, J., Ratnatunga, C., Pilla, R. and Channon, K. M. (2002). Mechanisms of increased vascular superoxide production in Human Diabetes Mellitus: Role of NAD (P) H Oxidase and Endothedial nitric oxide synthase. *Circulation*, 105:1656-1662.

Harborne, J.B. (1998). *Phytochemical methods*: A Guide to Modern techniques of plants Analysis. Chapman and Hall London, *UK*.

Harborne, J.B. (2005). Phytochemical methods. A guide to modern techniques of plant analysis, Chapman and Hall, London; pp.1.

Harinder, S. M. and Karen, L.G. (2001). Atorvastatin: An updated review of its Pharmacological properties and uses in Dyslipidaemia. *Adis drug Evaluation*, 61(12): 1835-1881.

Harris, W.S., Dujovne, C.A., Windsor, S.L., Gerrond, L.L.C., Newton, F.A. and Gelfand, R.A. (1997). Inhibiting cholesterol absorption with CP-88, 818 (beta-tigogenin cellobioside; tiqueside): studies in normal and hyperlipidemic subjects. *Journal of Cardiovascular Pharmacology*, 30: 55-60.

Haussament, T. U. (1977). Qualitative determination of serum alkaline phosphate. Clinical chemistry. *Acta*, 35:271-273.

Heber, M.F., Ferreira, S.R. and Velez, L.M. (2013). Prental hyperandrogenism and lipid profileduring different age stages: an experimental study. *Fertil steril*, 99 (2): 551-557.

Hetal, R. C. and Dion, R. B. (2013). The Single Dose Poloxamer 407 Model of Hyperlipidemia; Systemic Effects on Lipids Assessed Using Pharmacokinetic Methods, and its Effects on Adipokines. *Journal Pharmacy and Pharmaceutical Science*, 16(1): 65 – 73.

Hideo, O., Akio, M., Yasuko, Y., Mitsuru, N. and Yoshimasa, T. (1993) Antioxidant activity of tannins and flavonoids in *Eucalyptus rostrata*. *Phytochemistry,* 33:557–561.

Hollman, P.H. (1999). Dietary flavonoids: intake, health effects and bioavailability. *Food and Chemical Toxicology*, *37*(9): 937-942.

Hori, R. and Sugiyama, J. (2003). A combined FT-IR microscopy and principal component
analysis on softwood cell walls. *Carbohydrate Polymer*, 52: 449–453.

Horn, V.L. (1997). Fiber, lipids and coronary heart disease, a statement for healthcare professionals from the nutrition committee, American heart association. *Circulation,* 95: 2701-2704.

Hostettmann, K. and Marston, A. (1995). Saponins; Cambridge University Press: Cambridge, United Kingdom, New York.

Hou, R. and Goldberg, A.C. (2009). Lowering low-density lipoprotein cholesterol: statins, ezetimibe, bile acid sequestrants, and combinations: comparative efficacy and safety. *Endocrinology Metabolism Clinical North American,* 38: 79-97.

Hu, L.Z., Li, X.H., Ma, Y.Q. and Yu, X. (2011). Study on bacteriostatic activity of extracts
in different solventsfrom *Tephrosia vogelii* Hook f. seeds. *Science and Technology of Food Industry,*1, 13-18.

Huupponen, R.K., Viikari, J.S. and Saarim, H. (1984). Correlations of serum lipids with diabetes control in sulphonyhurea treated diabetic patients. *Diabetes Care*, 7: 575-578.

Hyeung, S., Hyo, C.A. and Sae, K. (2006). Hypolipemic effect of aqueous extracts of *Picrorrhi zarhizoma* in Poloxamer-407 induced hyperlipidemic mouse. *Journal of Ethnopharmacology*, 105: 380–386.

Ibrahim, B., M'batchi, B., Mounzeo, H., Bourobou, H.P. and Passo, P. (2000). Effect of *T. vogelii* and *Justicia extensa* on *Tilapia niloticain vivo*. Journal of Ethnopharmacology, 69: 99-104.

Igogo, J. M.(2013). Biological Activity of *Tephrosia Vogelii* Hook and *Lantana camara* L.
aqueous crude extracts against golden flea beetle (*Aphthonawhitfieldi* Bryant) in jatropha (*Jatropha curcas L.*)A Thesis submitted to the Graduate School in partial fulfilment for theRequirements of the Degree of Master of Science in Agronomy (CropProtection) of Egerton University.

Ikewuch, C.J. and Ikewuchi, C.C. (2010). Alteration of Plasma Lipid Profiles and atherogenic Indices by *Stachytarpheta jamaicensis L*. (Vahl). *Biochemistry*, 21, 71-77.

Inalegwu, B. and Sodipo, O. A. (2013). Phytochemical screening and haemolytic activities
of crude and purified saponins of aqueous and methanolic extracts of leaves of *Tephrosia vogelii* Hook. F. *Asian Journal of Plant Science and Research*, 3(5):7-11.

Inalegwu, B. and Sodipo, O. A. (2015).Antimicrobial and foam forming activities of extracts
and purified saponins of leaves of *Tephrosia vogelii*. *European Journal of Experimental Biology*, 5(5):49-53.

Jackson, J. E., Bressler, R. and Roa, D. (1999). Clinical pharmacology of sulphonylurea hypoglycemic agents: Part 1. *Drugs*, 22: 211-245.

Jacob, N. J. (2010). Spectrophotometric determination of Triacylglycerol in human serum. *Archive of Biochemistry Biophysics*, 88: 250-255.

Jacobsen, W., Kuhn, B. and Soldner, A. (2000). Lactonization is the critical first step in the disposition of the 3-hydroxy-3- methylglutaryl-CoA reductase inhibitor atorvastatin. *Drug Metabolism Disposition*, 28: 1369–1378.

Jain, G.C., Jhalani,S., Agarwal, S. and Jain, K.(2008). Hypolipidemic and antiatherosclerotic
effectof *Leptadenia pyrotechnica* extract in cholesterol fed rabbits. *Asian Journal of Experimental Science*, 21l (1):115-122.

James, D.B., Owolabi, O.A., Bisalla, M. and Jassium, H. (2010). Effects of aqueous extracts (leaves and stem) of *vitex doniana* on carbon tetrachloride induced liver injury in rats. *British Journal of Pharmacology and Toxicology*, *1*(1): 1-5.

Jananie, R.K., Priya, V. and Vijayalakshmi, K. (2011). Determination of Bioactive Components of *Cynodon dactylon* by GC-MS Analysis. *New York Science Journal*, 4 (4): 16-20

Jani, B. C. and Rajkumar, C. (2006). 'Ageing and Vascular Ageing'. *Postgraduate Medicine Journal*, 82:357-362.

Javed, I., Rahman, Z.U., Khan, M.Z., Muhammad, F., Aslam, B., Iqbal, Z., Sultan, J.I. and Ahmad,I. (2009). Antihyperlipidaemic efficacy of Trachyspermum ammiin albino rabbits.Acta. *Veterinary Brno*, 78:229-236.

Jegadeeswari, P., Nishanthini, A., Muthukumarasamya, S. and Mohan, V.R. (2012).Gc-Ms
analysis of bioactive components of *Aristolochia krysagathra* (Aristolochiaceae). *Journal of Current Chemical and Pharmaceutical Science*, 2(4); 226-232.

Ji, S. K., Jung, B. J., Chang, W. C. and Sei, C. K. (2006). Hypoglycemic and antihyperlipidemic effect of four korean medicinal plants in alloxan induced diabetic rats. *American Journal of Biochemistry and Biotechnology* 2 (4): 154-160.

Jiang, J. (2009). Polyunsaturated Fatty Acids and Obesity. *Application Clinical Pediatrics*,
24(19): 152-1524.

Johnson, M., Palmer, W.K. and Bradford, C. (2004). Omega-3, Omega-6 and Omega-9 Fatty
Acids: Implicationsfor Cardiovascular and Other Diseases. *Journal of Glycomics Lipidomics* 4: 123.0-41.

Johnston, T.P. and Palmer, W.K. (1993). Mechanism of poloxamer (407) induced hypertriglyceridemia in the rat. *Biochemical pharmacology*, *46*(6): 1037-1042.

Johnston, T.P. and Palmer, W.K. (2003). Mechanism of poloxamer 407-induced hypertriglyceridemia in the rat. *Biochemical Pharmacology*, 46, 1037–1042.

Johnston, T.P., Baker, J.C., Jamal, A.S., Hall, D.D., Emeson, E.E. and Palmer, W.K. (1999). Potential down regulation of HMG-CoA reductase after prolonged administration of Poloxamer (407) in mice. *Journal of Cardiovascular Pharmacology*, 34: 831–842.

Johnston, P.G. and Waxman, A. (2008). Flavonoids as antioxidants. *Journal of natural products*, *63*(7): 1035- 1042.

Jones, T.H., Morawetz, R.B. and Crowell, R.M. (1981). Thresholds of focal ischemia in awake monkeys. *Journal of Neurosurgery*, 54:773-782.

Jones, P. (1998). Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin and fluvastatin in patients with hypercholesterolemia (The CURVES study). *American Journal of Cardiology*, 81: 582-731.

Joseph, L.G., Helmut, G.S., William, R.H., Edwin, L.B. and Arno, G.M. (1973). Hyperlipidemia in Coronary Heart Disease. II. Genetic Analysis of Lipid Levels in 176 Families and Delineation of A New Inherited Disorder, Combined Hyperlipidemia. *Journal of Clinical Investment*, 52: 1544-1568.

Jousilahti, V. and Toumilehto, P. (1999). 'Sex, Age, Cardiovascular Risk Factors, and coronary heart disease'. *Circulation*, 99: 1165-1172.

Kalume, M.K., Losson, B., Angenot, L., Tits, M., Wauters, J.N., Frédérich, M. and Saegerman, C. (2012).Rotenoid content and in vitro acaricidal activity of *Tephrosia vogelii* leaf extract on the tick*Rhipicephalus appendiculatus*. *Veterinary Parasitology*, 190, 204-209.

Karmarkar, B. (2008). Poloxamers and their applications in Pharmacy Student Articles. Retrieved on 15th February, 2012 from http://www.pharmainfo.net

Kastelein, J.J.P., Holme, L., Gaffney, M., Cater, N.B., Barter, P., Deedwania, P., Olsson, A.G., Boekholdt, S.M., Demicco, D.A., Szarek, M., Larosa, J.C., Pedersen, T.R., Grundy, S.M., TNT and IDEAL study Groups (2008). Lipids apolipoproteins and their ratios in relation to cardiovascular events with statin treatment. *Circulation*, 117(23): 3002-3009.

Kato, N. and Tosa, N. (1983). *Journal of Agriculture Biology Chemistry* 47: (21) 19-20.

Keerthiga, M. and Anand, S. P. (2015).Bioactive Compound Evaluation of Ethanol Extract from *Geodorum densiflorum* (Lam.) Schltr. by GC-MS analysis, *International Journal of Pharmacological Research*, 5 (6): 136-144.

Kesäniemi, A. (2007). Cholesterol absorption inhibitors in the treatment of hyperlipidemia: clinical outcomes in large clinical trials. *Fundamental Clinical Pharmacology*, 21: 29-30.

Khallaf, M. (2011). *The Impact of Air pollution on Health, Economy, Environment and Agricultural sources*. In Tech. pp.69-92.

Khan, M.R. and Siddique, F. (2012). Antioxidant effects of *Citharexylum spinosum* in CCl4 induced nephrotoxicity in rat. *Experimantal Toxicology and Pathology*, 64:349-355.

Khush, K.K. and Waters, D.D. (2006). Effects of statin therapy on the development and progression of heart failure: mechanisms and clinical trials. *Journal of Cardiovascular Failure*, 12(8), 664-674.

Kim, H.Y., Jeong, D.M., Jung, H.J., Jung, Y.J., Yokozawa, T. and Choi, J.S. (2008). Hypolipidemic Effects of *Sophora flavescens* and its Constituents in Poloxamer 407-Induced Hyperlipidemic and Cholesterol-Fed Rats. *Biological and Pharmaceutical Bulletin*, 31, 73–78.

Kiotsis, D.N., Fillippatos, T.D., Mikhailidis, D.P. and Elisaf, M.S. (2007). Statin-associated adverse effects beyond muscle and liver toxicity. *Atherosclerosis*, 195(1): 7-16.

Kitagawa, I., Wang, H. K., Taniyama, T. and Yoshikawa, M. (1986). Saponin and sapogenol. XLI. Reinvestigation of the structures of soyasapogenols A, B, and E, oleanene-sapogenols from soybean. Structures of soyasaponins I, II, and III. *Chemical and Pharmacological Bulletn*, 36, 153-161.

Klaassen, C.D. (1996). Nonmetallic environmental toxicants; Air pollutants, solvents, vapors and pesticides. Pharmacological Basis of Therapeutics 9th edn. New York: McGraw-Hill.

Kobayashi, M., Ikegami, H., Fujisawa, T., Nojima, K., Kawabata, Y. and Noso, S. (2007). Prevention and treatment of obesity, insulin resistance, and diabetes by bile acid-binding resin. *Diabetes*, 56: 239-471.

Koh, K.K., Quon, M.J., Rosenson, R.S., Chung, W.J. and Han S.H. (2008). Vascular and metabolic effects of treatment of combined hyperlipidemia: focus on statins and fibrates. *International Journal of Cardiology*, 124: 149-591.

Kourounakis, A., Victoratos, P., Peroulis, N., Stefanou, N., Yiangou, M., Hadjipetrou, L. and Kourounakis, P.N. (2002). Experimental hyperlipidaemia and the effect of NSAIDs. *Experimental Molecular Pathology*, 73(2):135-138.

Kritchevsky, D. (1988). Cholesterol vehicle in experimental atherosclerosis. A brief review with special reference to peanut oil. *Archives Pathology Laboratory Medicine*, 112: 1041- 1047.

Kuczmarski, R.J., Flegal, K.M., Campbell, S.M. and Johnson, C.L. (1991). Increasing prevalence of overweight among US adults. The national health and nutrition examination surveys. *Journal of American Medicinal Association*, 272: 205–211.

Kumar, A., Agarwal, S., Heyman, J.A., Matson, S., Heidtman, M., Piccirillo, S., Umansky, L., Drawid, A., Jansen, R., Liu, Y., Cheung, K.H., Miller, P., Gerstein, M., Roeder, G.S. and Snyder, M. (2002). Types of primary hyperlipidemia. *Nutrition Research*, 16**:** 707–719.

Kumar, A. R.I., Lavarasan, T., Jayachandran, M., Decaraman, P., Aravindhan, N., Padmanabhan, G. and Krishnan, M.R.V. (2009). Phytochemical investigation on a tropical plant, *Syzygium cumini* from Kattuppalayam, Erode District, Tamil Nadu, chupandia South India. *Pakistan Journal of Nutrition*, 8: 83-85.

Kumar, S., Kumar, V. and Prakash, O. (2010). Antidiabetic, hypolipidemic and histopathological analysis of Dillenia indica (L.) leaves extract on alloxan induced diabetic rats. *Asian Pacific Journal of Tropical Medicine*, 4(5): 347-352.

Laakso, M. (1999). Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes*, 48(5):937-947.

Law, M.R., Wald, N.J. and Rudnicka, A.R. (2003). Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *Biology Medicine Journal*, 326 (4):1423.

Lee, S.H.; Jeong, T.S., Park, Y.B., Kwan, Y.K., Choi, M.S. and Bok, S.H. (1999). *Nutrition*
Reserve. **19**: (12) 45-58.

Lee, S.I., Oh, S.H., Park, K.Y., Park, B.H., Kim, J.S. and Kim, S.D. (2009). Antihyperglycemic effects of fruits of privet (*Ligustrum obtusifolium*) in streptozotocin induced diabetic rats fed a high fat diet. *Journal of Medicinal Food*, 12:109-171.

Leeder, S., Raymond, S. and Greenberg, H. (2004). Race against time: The challenge of cardiovascular disease in developing economies. New York: Columbia University.

Li, X.H., Huang, X.L., YU, X., Zhu, X.Y. and Huang, X.H. (2010). Study on scavenging free radial activity ofextracts from *Tephrosia vogelii* Hook f. Seeds. *Food Research and Development*,1, 5-9.

Libby, C., Saad, B. and Saed, S. (2000). Safety of traditional Arab herbal medicine. *EvidenceBased Complementary and Alternative Medicine*, 3: 433-439.

Lim, S., Vos, T., Flaxman, A. D., Danaei, G., Shibuya, K. and Adair-Rohani, H. (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for theGlobal Burden of Disease Study 2010. *Lancet*, 2012.

Liu, H., Sun, S., Lv, G. and Chan, K.K.C. (2006). Study on Angelica and its different extracts by Fourier transform infrared spectroscopy and two-dimensional correlation IR spectroscopy. *Spectrochimica Acta Part A*, 64: 321–326.

Lorke, D. (1983). *A New Approach to Practical Acute Toxicity Testing*. Arch Toxical. pp. 275-287.

Lu, H. F., Cheng, C.G., Tang, X. and Hu, Z.H. (2004). Spectrum of *Hypericum* and *Triadenum* with reference to their identification. *Acta Botanica Sinica*, 46:401-406.

Lu, Q. y., Arteaga, J.R., Zhang, Q., Huerta, S., Go, V.L. and Heber, D. (2005). Inhibition of prostate cancer cell growth by an avocado extract: Role of lipid-soluble bioactive substances. *Journal of Nutritional Biochemistry*, 16:23-30.

Lu, L.S., Wu, C.C., Hung, L.M., Chiang, M, T., Lin, C.T., Lin, C.W. and Su, M.J. (2007). Apocynin alleviated hepatic oxidative burden and reduced liver injury in hypercholesterolaemia. *International Journal of Biomedical Toxicology*, 27:529-537.

Luiz, C., Bertges, 1. Carlos, A. and Mourao, J. (2011).Hyperlipidemia induced by triton WR1339 (tyloxapol) in Wistar rats. *Review Bras Cien Medicine Saúde*, 1; 132-134.

Ma, J., Qiao, Z. and Xiang, X. (2011). Aqueous extract of *Astragalus mongholicus* ameliorates highcholesterol diet induced oxidative injury in experimental rats' models. *Journal of Medicinal Plants*, *5*: 855–858.

Machocho, A.K., Lwande, W., Jondiko, J.L., Moreke, L.V. C. and Hassanali, A. (1995). Three new flavonoids from the roots of *Tephrosia. Emoroides*and their antifeedant activity against larvae of the Spotted stalk borer, *Chilo Partellus* Swinhoe.

International Journal of Pharmacognosy, 33: 222-227.

Mackay, C., Mensal, B. and Mendis, K. (2004). The Atlas of Heart Disease and Stroke. World Health Organisation.

Mahfouz, M.M., H. Kawano, K. and Kummerow, A.K. (1997). Cholesterol-rich diets have different effect on lipid peroxidation, cholesterol oxides and antioxidant enzyme in rats and rabbits. *American Journal of Clinical Nutrition,* 66: 1240-1249.

Mahmood, Z.A., Mohammed, S., Mahmood, S.B.Z. and Karim, M.A. (2010). Herbal treatment for cardiovascular disease the evidence based therapy. *Pakistan Journal of Pharmaceutical Sciences*, 23(1):119-124.

Mahmoud H. H. (2016). New method for assessment of serum catalase activity.*Indian Journal of Science and Technology*, 9(4); 10

Makoshi, M. S. and Arowolo, R. O. A. (2011). Therapeutic effects of *Tephrosia vogelii* ointment in the treatment of bovine dermatophilosis.*Journal of Veterinary Medicine and Animal Health*, 3(4) 51-55.

Malinon, R. (1997). Blood cholesterol lowering properties of dietary Saponins. *American Journal of Clinical Nutrition*, 6: 42-45.

Manonmani, R. and Catharin, S. (2015). GC-MS Analysis of Bioactive Components of an
ImportantMedicinal Fern *Actiniopteris radiata* (Swartz) Link. *World Journal of Pharmaceutical Research*, 4(4): 60-69.

Marja, P.K., Anu, I.H., Heikki, J.V., Jussi-Pekka, R., Kalevi, P., Tytti, S.K. and Marina, H.J. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural Food Chemistry*, 47:3954–3962.

Marshall, A. F. (1992). Flavonoids and auxin transport: modulators or regulators. *Trends in plant Science,* 12: 556-563.

Martin, J.P., Dailey, M. and Sugarman, I. (1987). Negative and Positive Assays of superoxide Dismutase based on Heamatoxylin Autoxidation. *Archives of Biochemistry and Biophysics*, 255:329-336.

Martinez, F.J., Rizza, R.A. and Romero, J.C. (1994).High fructose feeding elicits insulin resistance, hyperinsulinemia and hypertension in normal mongrel dog. *Hypertension,* 23:456-463.

Massimo, F. and Mannuccio, P. M. (2011). Air pollution and cardiovascular disease, *Thrombosis Research*, 21:49-84.

Mathew, O.W., and Blessing, C.D. (2007). Hepatoprotective effects of Garcinia kola seed
against hepatotoxicity induced by carbon tetrachloride in rats. *Biochemistry*, 19(1): 17-21.

Maton, A. (1993). *Human Biology and Health. Englewood Cliffs*, New Jersey: Prentice hall.ISBN 0-13-981176-1.

Matovu, H.andOlila, D. (2007).Larvicidalactivityof*Tephrosiavogelii*crude extracts onmosquito larvalstages. *ResearchJomnal ofBiologicalSciences,*2(6):612-616.

Matthijsen, J. and Brink, H.M. (2007). $PM_{2.5}$ in the Netherlands, consequences of the new
European Air quality standards, Netherlands Environmental Assessment Agency (MNP), Bilthoven, The Netherlands, Report 500099001.

Mbomi, S.E., Ogungbesan, A.M., Babayemi, O.J. and Nchinda, V.P. (2011). Chemical composition, acceptabilityof three *Tephrosia* species and use of *Tephrosia purpurea* as supplement for grazinganimals in the western highlands of Cameroon. *Journal of Environmental Issues and Agriculturein Developing Countries*,3, 132-139.

McCullough, P.A., Ahmed, A.B., Zughaib, M.T., Glanz, E.D. and Di Loreto, M.J. (2011) Treatment of hypertriglyceridemia with fibric acid derivatives: impact on lipid subfractions and translation into an education in cardiovascular events. *Review Cardiovascular Medicine,* 12:173-85.

McDonnell, W.F., Nishino-Ishikawa, N., Petersen, F.F., Chen, L.H. and Abbey, D.E. (2000).
Relationship of mortality with the fine and coarse fractions of long term ambient PM10 concentrations in nonsmokers.*Journal Exposure of Analysis Environmental Epidemiology*, 10, 427-436.

McKenney, J.M., Koda-Kimble, M.A. and Young, L.Y. (2001). Dyslipiemias. In *Seven E*ditions.Lippincott Williams and Wilkins: Philadelphia, PA, USA *Applied Therapeutics*; pp. 11:1–11:43.

Megalli, S., Fugen, A., Neal, M. and Basil, D. (2005). Phytopreventive anti-hyperlipidemic effects of *Gynostemma pentaphyllum* in rats. *Journal of pharmacological and Pharmaceutical Science*, 8(3): 507-515.

Meenakshi, V.K., Gomathy, S., Senthamarai, S., Paripooranaselvi, M. and Chamundeswari,
K.P. (2012). GC-MS Determination of the Bioactive Components of *Microcosmus*
*exasperates*Heller, 1878. *Journal Current Chemical Pharmaceutical Science*, 2(4): 271-276.

Mendis, S., Puska, P. and Norrving, B. (2011). Global Atlas on cardiovascular disease prevention and control, ISBN 9789241564373.

Messina, M.J. (1999). Legumes and soybeans: Overview of their nutritional profiles and health effects. *America Journal of Clinical Nutrition*, 70(3): 439-450.

Middlemiss, D. and Watson, S.P. (1994). A medicinal chemistry case study: an account of an Angiotensin II antagonist drug discovery programme. *Tetrahedron*, 50:13-80.

Miraglia, S.G.K. and Gouveia, N. (2014). Custos da poluição atmosféricanas regiões metropolitanas brasileiras. *Cien SaudeColet,* 19(10):4141-4147.

Mishra, A.K., Mishra, A., Kehri, H.K., Sharma, B. and Pandey, A.K. (2009). Inhibitory activity of Indian spice plant *Cinnamomum zeylanicum* extracts against *Alternaria solani* and *Curvularia lunata* pathogenic dematiaceous moulds. *Annals for Clinical Microbiology and Antimicrobial*, 8: 9-10.

Moghimi, S.M., Hunter, A. C., Dadswell, C.M., Savay, S., Alving, C. R. and Szebeni, J. (2004). Causative factors behind poloxamer 188 (Pluronic F68, Flocor)-induced complement activation in human sera. A protective role against poloxamer-mediated complement activation by elevated serum lipoprotein levels. *Biochemical and Biophysics Acta,* 1689(2):103-131.

Mohan, C.U. (2011). Antihyperlipidemic effect of *Tephrosia calophylla* against acute and
chronichyperlipidemia in wistar albino rats. Post graduate thesis.

Murray, C.J. and Lopez, A.D. (1996). In: Global health statistics and Global burden of disease and injury series. Cambridge, Harvard University Press, 64–67.

Murphy, S.A., Cannon, C.P. and Braunwald, E. (2007). Effect of intensive lipid lowering therapy on mortality after acute coronary syndrome. *The American Journal of Cardiology,* 100(7):1047-1051.

Murugesan, A. and Shanmugam, R. (2014). Assessing the bioactive constituents of *cadaba*
fruticosa(l.) Druce through gc-ms *International Journal of Pharmacy and Pharmaceutical Sciences, 6, (2), 383-385*

Muthu, G.P., Balasubramanian, R., Raju, S. K., John, W. E., Ekambaram, P. K., Mani, R, K., Kunchu, K., Mohanraj, P., Nicholls, S.J., Tuzcu, E.M. and Sipahi, I. (2008). Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis. *Journal of the American Medical Association,* 297(5): 499-508.

Nash, V.J., Johnston, T.P. and Palmer, W. K. (1996). Effects of nicotinic acid on Poloxamer 407-induced hyperlipidemia. *Pharmacotherapy*, 16(1):10-15.

Nash, S.D. and Nash, D.T. (2008).Nuts as part of a healthy cardiovascular diet. *Current Atherosclerosis Rep,* 10:529-535.

National Cholesterol Education Program (NCEP) Expert Panel. (2001). Third report of the NCEP expert panel on detection, evaluation and treatment of high blood cholesterol in adults (ATP 111) NIH Puplication.Bethesa; National Heart, Lung and Blood Institute.

Nayak, B.S., Raju, S.S. and Chalapathi, R.A.V. (2008). Wound healing activity of *Persea americana*(avocado) fruit: a preclinical study on rats. *Journal of Wound Care,* 17:123-126.

Nicholls, S.J., Tuzcu, E.M. and Sipahi, I. (2007). Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis. *Journal of the American Medical Association,* 297(5): 499-508.

Nissen, S.E., Tuzcu, E.M. and Brodie, B. (2004). Effect of intensive compared with moderate lipid lowering therapy on progression of coronary atherosclerosis. *Journal of American Medical Association,* 291(9): 1071-1080.

Norazlina, M., Lee, P.L., Lukman, H.I., Nazrun, A.S. and Ima-Nirwana, S.(2007). Effects
ofvitamin E supplementation on bone metabolism in nicotine-treated rats.*Singapore Medicine Journal,* 48(3):195–199.

Norman, R., Bradshaw, D., Steyn, K., Gaziano, T. and Laubscher, R. (2006). Estimating the Burden of High Cholesterol in South African. *Medical journal*, 97: 692-698.

Nworgu, Z.A., Onwukaeme, D.N., Afolayan, A.J., Amaechina, F.C. and Ayinde, B.A. (2008). Preliminary studies of blood pressure lowering effect of *Nauclea latifolia* in rats. *African Journal of Pharmaceutical and Pharmacology*, 2:37-41.

Nwude, N. (1997).Ethno veterinary pharmacology and ethnoveterinary practices in Nigeria;
an overview. *Tropical Veterinary*, 15, 17-23.

Obdoni, B.O. Ochuko, P.O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science,* 8b:203-208.

Ock, K.C., Dae-Ok, K. and Chang, Y.L. (2003). Superoxide radical scavenging activity. *Journal of Clinical Pathology, 2*(12): 240-242.

Ojiakor, A. and Nwanjo, H. (2005). Effect of vitamine E and C on exercise induced oxidative stress. *Global Journal of Pure and Applied Sciences,* 12(2): 199-202.

Olaniran, O. A., Alao, F. O. and Adebayo, T. A. (2013). Control of foliage pests of roselle (*Hibiscus Sabdariffa* L.) using plant extracts of *Tephrosia vogelii* And *Azardiractha indica* In Ogbomoso, Nigeria. *Transnational Journal of Science and Technology*, 3 (6): 50-62.

Oleszeke, W. (1988). Solid phase extraction: Fractionation of alfaalfa saponins. *Journal of Science food Agriculture*, 44: 43 - 49.

Ornish, D. I. and Rosner, B. (2005). The bronchodilator effects of *Garcinia kola. East African Journal Medical Journal,* 70(3): 143-145.

Owolabi, J., Omogbai, E.K. and Obasuyi, O. (2007). Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelia africana* (Bignoniaceae) stem bark. *African Journal of Biotechnology*, 6:88-92.

Oyedemi, O.S., Bradley, G. and Afolayan, A.J. (2010). *In-vitro* and *in-vivo* antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. *Africa Journal of Pharmacology,* 4: 70–78.

Oyewole, J.A. and Amabeoku, G.J. (2006). Anticovulsant effect of *Persea americana*Mill
(Lauraceae) (Avocado) leaf aqueous extract in mice. *Phytotherapy Resource*, 20:696-700.

Ozbek, H., Ozturk, M., Ozturk, A., Ceylan, E. and Yener, Z. (2004). Determination of Lethal
Doses of Volatile and Fixed Oils of Several Plants. *Eastern Journal of Medicine,* 9 (1): 4 – 6.

Palmer, W.K., Johnston, T.P., Baker, J.C., Jamal, A.S., Hall, D.D. and Emeson, E.E.(1983). Potential down regulation of HMG-CoA reductase after prolonged administration of Poloxamer (407) in mice. *Journal of Cardiovascular Pharmacology,* 34: 831–842.

Pandey, A. and Singh, P. (2011). *Asian Journal of Plant Science and Research,* 1 (2):69-80.

Pankaj, N., Karan, D. and Sacchidanandmani, T. (2011). Study of antihyperlipidemic effect
on the juice of the fresh fruits of *Lagenaria siceraria. International Journal of Pharmacy and Pharmaceutical Sciences*, 3(1): 3-9.

Parra, J.L. and Reddy, K.R. (2003). Hepatoxicity of hypolipidemic drugs. *Clinical Liver*,

7(2): 415-433.

Patel, D.K., Patel, K.A., Patel, U.K., Thounaoja, M.C., Jadeja, R.N., Ansarullah, P.G.S., Salunke, S.P., Devkar, R.V. and Ramachandra, A.V. (2009). Assessment of lipid lowering effect of *Sida rhomboidea* methanolic extract in experimentally induced hyperlipidemia. *Journal of Pharmaceutical sciences*, 1: 233–238.

Paul, J. P. M., Yesu, R.j., Prabhu, S. V.P., Joy, V., Saravanan, J. and Sakthivel, S. (2012).
GC-MS Analysis of bioactive components on the Leaves extract of *Stylosanthes fruticosa*- A potential folklore medicinal plant. *Asian Journal of Plant Science and Research,* 2 (3): 243-253.

Pavana, P., Manoharan, S., Renju, G.L. and Sethupathy, S. (2007). Antihyperglycemic and
antihyperlipidemiceffects of *Tephrosia purpurea* leaf extract in streptozotocin induced diabetic rats. *Journal ofEnvironmental Biology,*28, 833-837.

Pomulo, R.N.A. and Ierece, L.R. (2007). MiR-27b attenuates apoptosis induced by transmissible gastroenteritis virus (TGEV) infection via targeting runt-related transcription factor 1 (RUNX1).*Bioscience*, 57 (11): 949 -955.

Pope, A. C., Burnett, R.T., Thun, M.J., Calle, E.E., Krewski, D., Ito, K. and Thurston, G.D.
(2002). Lung cancer, cardiopulmonary mortality and long-term exposure to fine particulate air pollution, *JAMA* 287, 1132-1141.

Potter, S.M., Jimenez-Flores, R., Pollack, J., Lone, T.A. and Berber-Jimenez, M.D. (1993). Protein-saponin interaction and its influence on blood lipids. *Journal of Agriculture and Food Chemistry,* 41: 1287-1291.

Price, K.R., Johnson, T. and Fenwick, G.R. (1987). The Chemistry and biological significance of saponins in food and feeding stuffs, *Critical Pure Applied Science*, 8b:203-208.

Prohp, T. P. and Onoagbe, I. O. (2012). Acute toxicity and dose response studies of aqueous
and ethanol extracts of *Triplochiton scleroxylon* K. schum, (sterculiaceae).
International Journal of Applied Biology and Pharmaceutical Technology, 3(1): 400-409.

Pulsinelli, W.A. (1995).The ischemic penumbra in stroke. *Science Medicine,* 1:16-25.

Rajani, G.P. and Ashok, P. (2009). *In vitro* antioxidant and antihyperlipidemic activities of *Bauhinia variegata* Linn. *Indian Journal of Pharmacology*, 41, 227–232.

Rajeswari, B. and Muthuirulappan, S. (2015). GC-MS Analysis of bioactive components
from the ethanolic leaf extract of *Flueggea leucopyrus*Wild, *International Journal of Pharmaceutical Sciences Review and Research,* 33(1), 50, 270-273.

Ramaiah, S.K. (2007). Reflection paper on non-clinical evaluation of drug-induced liver
injury (DILI)Food ChemTox, 45, 1551.

Ranjan, S., Dasgupta, N., Saha, P., Rakshit, M. and Ramalingam, C. (2012). Advances in *Applied Science Research*, 3 (1):495-501.

Ranjna, C. (1999). *Practical Clinical Biochemistry Methods and Interpretation*. 2nd edn, Jaypee Brother Medical Publishers (P) Limited Nov. Delhi, India. p. 117.

Rashid, S.A., Halim, A.S., Muhammad, N.A. and Suppl, A. (2008). The effect of vitamin E onbasic fibroblast growth factor level in human fibroblast cell culture.*Medicine Journal of Malaysia*, 63:69–70.

Ray, K. K., Cannon, C. P. and Ganz, P. (2006). Beyond lipid lowering: what have we learned about the benefits of statins from the acute coronary syndromes trials? *American Journal of Cardiology*, 98(11): 18-25.

Reitman, S. and Frankel, A.S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Serum glutamic oxaloacetic and glutamic pyruvic transaminase. *American Journal of Clinical Pathology*, 28:56-61.

Ridker, P.M., Genest, J., Boekholdt, S.M., Libby, P. and Gotto, A.M. (2010). HDL cholesterol and residual risk of first cardiovascular events after treatment with potent statin therapy: an analysis from the JUPITER trial. *Lance,t* 376: 333-912.

Robert, k. M., Daryl, K. G. and Victor, W. R. (2006). Harper's illustrated Biochemistry 27th Ed McGraw-Hill Education, Asia.

Romero, F.J., Bosch-Morell, F., Romero, M.J., Jaren, E. J., Romero, B. and Marı, N. (1998). Lipidperoxidation products and antioxidants in human disease. *Environ Health Perspect*, 106: 1229–1234.

Rosuvastatin. (2003)-a new lipid-lowering drug. *Medicine Lett Drugs Therapy*, 45: 81-83.

Saad, B., Azaizeh, H., Abu-Hijleh, G. and Said, S. (2006). Safety of traditional Arab herbal medicine. *Evidence Based Complementary Alternative Medicine*, 3: 433-439.

Saha, S.A., Kizhakepunnur, L.G. and Bahekar, A. (2007). The role of fibrates in the prevention of cardiovascular disease-a pooled meta-analysis of long-term randomized placebo-controlled clinical trials. *American Heart Journal*, 154(5):943.

Sahoo, S., Chakraborti, C.K., Mishra, S.C, Nanda, U.N. and Naik, S. (2011). FTIR and XRD Investigations of some Fluoroquinolones; *International Journal*

Pharmacology Science; 3(3): 165-170.

Sathiyabalan, G.1., Packia L. M.1., Muthukumarasamy, S. and Mohan, V.R. (2014). GC-
MS Analysis of Bioactive Components of *Petiveria alliacea* L. Whole Plant (Phytolaccaceae) *International Journal of Pharmacology Research and Health Sciences,*2 (5), 387-392.

Sathya, S. Srisudha1, S. and Gunasekaran, P. (2010). Growth rate, pigment composition and fatty acid profile of chlorella pyrenoidosa. *International Journal of biological and pharmaceutical research*, 3(5): 677-683.

Saurav, A., Kaushik, M. and Mohiuddin, S.M. (2012). Fenofibric acid for hyperlipidemia. *Expert Opin Pharmacother*; 13: 717.

Schafer, S.H., Hussein, G.R., Gershony, J.C. and Kappagoda, C.T. (1997). Regression of
severe atherosclerotic plague in individuals with mild elevation of LDL cholesterol. *Journal of Internal Med*icine, 45: 536-541.

Schmolka, I.R. (1991). *Poloxamers in the pharmaceutical industry*. In: Tarch PJ, ed. Polymers for controlled drug delivery. Boca Raton: CRC Press: 189-215.

Shah, K.A., Mandev, B. P. and Shreya, S. (2010). Antihyperlipidemic activity of *Mangifera indica*. Leaf extract on rats fed with high cholesterol diet. *Der Pharmacia Sinica,* 1(2): 156-161.

Sheneni, V. D., James, D. B. and Atawodi, S. E. (2014). Effect of ethanolic extract of *Vitex donian* on hepatocuractive property in poloxamer induced hyperlipidemia. *Science Research.* 2(3): 49-54.

Sheyla, L. M., Heberth, P., Maria, L., Pedrosa, R. C. and Santos, E. (2005). Dietary models
for inducing hypercholesterolemia in Rats. *An International Journal Brazilian Archives ofBiology and Technology*, .48, (2): 203-209.

Shibata, A., Nakagawa, K., Sookwong, P., Tsuduki, T., Tomita, S., Shirakawa, H.,Komai,
M. and Miyazawa, T. (2008). Tocotrienol inhibits secretion of angiogenicfactors from human colorectal adenocarcinoma cells by suppressinghypoxia-inducible factor-1alpha. *Journal of Nutrition*, 138(11):2136–2142.

Siamba, D.N., Okitoi, L.O., Watai, M.K., Wachira, A.M., Lukibisi, F.B. and Mukisira, E.A.
(2007). Efficacy of*Tephrosia vogelli* and *Vernonia amygdalina* as anthelmintics against*Ascaridia galli* inindigenous chicken. *Livestock Research for Rural Development,*19, 12.

Sidhu, G.S. and Oakenfull, D.G. (1986). A mechanism for the hypocholesterolaemic activity of saponins. *British Journal of Nutrition,* 55, 643-649.

Smelt, A.H. (2010). Triglycerides and gallstone formation. *Clinical Chim Acta,* 411: 1625-31.

Smith, S.C. (2004). Heart diseases prevention must get more attention. World Health Federation, *Circulation*, 1: 2.

Smith I.F. and Eyzaguine P.B. (2007). African leafy vegetables: Their role in the World Health Organization‟s Global Fruit and Vegetable Initiative. *African Journal of Food Agriculture and Nutrition Development,* 7(3): 1-17.

Soetan, K.O. (2008). Pharmacological and other beneficial effects of antinutritional factors in plants-A review. *African journal of Biotechnology*, *7*(25): 55-60.

Sofowara, A. (1993). *Medicinal Plants and Traditional Medicine in Africa*. Spectrum books Ltd., Ibadan, Nigeria. P. 289.

Soko, U. (1999). Chemopreventive activity of plants. *Journal of Science Americana,* 270(6) : 82.

Sowmya, A. and Ananthi, T. (2011). Hypolipidemic activity of *Mimosa pudica* Linn on butter inducedhyperlipidemia in rats.*Asian Journal of Reserve Pharmacuetical Science,* 1(4):123-126.

Spencer, C. O. N., Sunday, J. J., Teslimat, E. A., Kazeem, O. A., Eguagie, O. O. andAkinola,
A. A. (2011). Comparative effects of aqueous and ethanolic leaf extractsof *Gongronema latifolium* on serum kidney and liver biomarkers of normal male rats. *Asian Journal of Biological Science*, 4: 540-547.

Stacy, T.A. and Egger, A. (2006). Results of retrospective chart review to determine improvement in lipid goal attainment in patents treated by high-volume prescribers of lipid-modifying drugs. *Journal of Management Care Pharm*acy, 12(9): 745-751.

Stein, E.A., Ballantyne, C.M. and Windler, E. (2008). Efficacy and tolerability of fluvastatin XL 80mg alone, ezetimibe alone, and the combination of fluvastatin XL 80mg with ezetimibe in patients with a history of muscle-related side effects with other statins. *American Journal of Cardiology,* 15 (4):490-510.

Steinberg, D. and Witztum, J. L. (2011). Oxidized low density lipoprotein and atherosclerosis. *Arteriosclerosis Thrombus Vascular,* 30:2311-2316.

Stone, N.J. (1994). Secondary causes of hyperlipidemia. *Medicinal Clinical North America*, 78: 117-119.

Sujatha, K., Sivakamasundari, M. and Chandramohan, G. (2014). GC-MS analysis of Phytocomponents and Total Antioxidant Activity of Hexane Extract of *Sinapis Alba*. *International Journal of Pharmaceutical Chemical and Biological Sciences*; 4(1): 112-117.

Suleiman, M. S., Zolieka, M. and Durdi, Q. (2012). Hypolipidemic effects of ethanolic and aqueous extracts of *Urtica dioica*in Rats. *World Applied Sciences Journal,* 17 (10): 1345-1348.

Sundaram, M. and Yao, Z. (2010). Recent progress in understanding protein and lipid factors affecting hepatic VLDL assembly and secretion. *Nutritional Metabolism,* 7: 35-41.

Suzuki, T. and Suzuki, Y. (2006). *Biology Pharmaceutical Bull*, 29: 1538.

Syed, S., Ghori, 1. M.A., Rizwan, K., Kaab, A. and Hussain, A. (2015).Evaluation of antihyperlipidemic activity of ethanolic extact of *Glycosmis pentaphylla* in hyperlipidemic wistar rats.*International Journal of Pharma Sciences and Research,* 6 (2): 282-292.

Takahama, S. and Dillner, A. M. (2015). Model selection for partial least squares calibration andimplications for analysis of atmospheric organic 35 aerosol samples with mid-infrared spectroscopy, *Journal of Chemometrics*, 29, 659–668.

Teugwa, M. C., Sonfack D. C. R., Fokom, R., Penlap, B. V. and Amvam, Z. P. H. (2013). Antifungal and Antioxidant Activity of Crude Extracts of Three medicinal plants from Cameroon pharmacopea. *Journal of Medicinal Plants Research,* 7(21): 1537-1542,

Thirupathy, S., Malayaman, V., Sisubalan, N. and Ghouse, B. M. (2014). Antibacterial activity of *Tephrosia Hookeriana* Wight and Arn Leaf Explants *International Journal of Applied Biology and Pharmaceutical Technology* 5 (1): 85-89.

Thomas, S. and Rich, M.W. (2007). Epidemiology, Pathophysiology, and prognosis of heart failure in the elderly. *Clinical Geriatric Med*icine, 23(1): 1-10.

Thorburn, A.W., Storlein, L.H., Jenkins, A.B., Khouri, S. and Kraegen, E.W. (1989). Fructose induced *in vivo* insulin resistance and elevated plasma triglyceride levels in rats. *American Journal of Clinical and Nutrition,* 49:1155-1163.

Tolins, C., Miller, B. and Keane, W.F. (1992). Lipid-induced glomerular injury.*Nephron*, *67*(1): 1-6.

Trible, D.L. and Jones, D.P. (1987). The pathophysiological significance of lipid peroxidation in oxidative cell injury. *Hepatology*, 7: 377-387.

Trifiro, G. (2006). Drug- Drug interactions and statin therapy. *South Medicine*, 99(12): 1325-
1326.

Usha, M. G., Duan, S. Z., and Mortensen, R. M. (2008). Liver function parameters. *Current opinion in hepathology*, *18*(2): 128-133.

Vaishwana, I. and C.N. Kowale, (1976). CNS effects of two Ayurvedic drugs Shilajeet and Edinolon changes in liver and serum lipids produced by carbon tetrachloride. *Indian Journal of Experimental Biology,* 14: 58-61.

Vance, E. and Vance, J.E. (2002). *Biochemistry of Lipids, Lipoprotein and Membranes*, fourth Edition. Elsier Science B.V. pdf

Vasconcelos, J. N., Lima, J.Q., Lemos, T.L.G.D., Oliveira, M.D.C.F.D., Almeida, M.M.B., Andrade-Neto, M. and Braz-Filho, R. (2009). Chemical and biological study of the *Tephrosia toxicaria* Pers. *QuímicaNova* 32, 382-386.

Vildan, A., Sülünay, P., Hüseyin, T. and Ercin, E. (2009). Synthesis and antimicrobial activity of Some Pyridinium Salts. *Molecules*; 14: 203-215.

Wacnic, R.G. and Alber J.J. (1978). A comprehensive evaluation of the heparin manganese precipitation procedure for estimating high density lipoprotein cholesterol. *Journal of Lipid Research*, 19: 65-76.

Wanga, B.N., Akenga, T., Imbuga, M., Gitonga, L., Olubayo, F. and Namungu. P. (2006). Antimicrobialacitivity of extracts from *Tephrosia vogelii* Hook F. *Journal of Agriculture, Science and Technology,* 8(1): 1-14.

Wexler, B.C. and Greenberg, B, P. (1978). Clofibrate retardation of naturally-occurring arteriosclerosis in repeatedly-bred male and female rats. *Atherosclerosis,* 29: 329-344.

Wickelgren, I. (1998). Obesity: how big a problem. *Sci*ence, 280: 1364–1371.

Wilt, T.J., Bloomfield, H.E. and MacDonald, R. (2004). Effectiveness of statin therapy in adults with coronary heart disease. *Archives International Medicine,* 12; 164 (13):1427.

World Health Organization. (1994). Joint WHO/ISFC meeting on RF/RHD control with emphasis on primary prevention, Geneva, 7–9.

World Health Organization. . (1997). World Health Report. Conquering suffering. Enriching
humanity. Geneva, 43–44.

World Health Organization. (1998). *Obesity: Preventing and Managing the Global Epidemic*. *Report of a WHO Consultation on obesity*, WHO/NUT/NCD/98, Geneva, Switzerland.

World Health Organization. (2015). Cardiovascular diseases, fact sheet #317. Retrieved from
http://www.who.int/mediacentre/factsheets/fs317/en/

Wright, A.D., Kong, G.M., Angerhofer, C.K., Greenidqe, P., Linden, A. and Desqueyroux-

Faundez, R. (1996). Antimalarial activity: the search for marine-derived natural productswith selective antimalarial activity. *Journal of Natural Product*, 59(7): 710-716.

Wu, J., Zhu, Y.H. and Patel, S.B. (1999). Cyclosporin-induced dyslipoproteinemia is associatedwith selective activation of SREBP-2. *American Journal of Physiology*, 277: 1087-1094.

Wu, S.J., Liu, P.L. and Ng, L.T. (2008). Tocotrienol-rich fraction of palm oil exhibits anti-
inflammatory property by suppressing the expression of inflammatory mediators in humanmonocytic cells. *Molecular Nutrition Food Resources,* 52(8):921–929.

Yadav, J.P., Saini, S., Kalia, A.N. and Dangi, A.S. (2008). Hypoglycemic activity of ethanolic extract of *Salvadora oleoides* in normal and alloxan induced diabetes rats. *Indian Journal of Pharmacol*ogy, 40:237.

Yang, X., Yang, L. and Zheng, H. (2010). Hypolipidemic and antioxidant effect of mulberry (*Morus Alba* L.) fruit in hyperlipiemia rats. *Food chemical Toxicology*, 48, 2374-2379.

Yokoyama, M., Origassa, H. and Matsuzawa, Y. (2010). Effects of eicosapentaeonic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomized open-label, blinded endpoint analysis. *Lancet*, 79:1- 49.

Yokozawa, T., Cho, E.J. and Sasaki, S. (2006). The protective role of Chinese prescription
kangen-karyu extract on diet- induced hypercholesterolemia in rats. *Biological and Pharmaceutical Bulletin,* 29: 760-765.

Young, S. L., Young, W. K., Sang, G. K., Inchul, L., Myung, G. L. and Hee, E. K. (2012). Effects of poloxamer 407-induced hyperlipidemia on the pharmacokinetics of carbamazepine and its 10, 11-epoxide metabolite in rats: Impact of decreased expression of both CYP3A1/2 and microsomal epoxide hydrolase. *European Neuropsychology pharmacology*, 22(6): 431-440.

Zalba, G., San, J.G., Moreno, M.U., Fortuno A., Beaumont F.J. and Diez J. (2001). Oxidative stress in arterial hypertention. Role of NADPH oxidase. *Hypertension*, 38: 1395–1399.

Zheng, W. and Wang, S.Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural Food Chemistry*, 49: 5165–5170.

Zobaer, A. M., Sitesh, C. B. and Nazmul, Q. (2011). Antihyperlipidemic activity of leaf and root extracts of Premnaesculenta (Roxb.) in Poloxamer-407 induced hyperlipidemic mice and rats. *Orient Pharmacological Experimental Medicine*, 11:263–270.

APPENDICES

Appendix 1

Quantitative Analysis of Active Ingredients of Aqueous Leaves Extract of *Tephrosia vogelii* (%)

Parameter	Saponins	Alkaloids	Flavonoids	Phenols
Leaves	9.00 ± 0.12^{d}	0.52 ± 0.23^{b}	0.62 ± 0.02^{b}	0.04 ± 0.01^{a}

Values are means $\pm$ SD of four determinations. Values with different superscripts down the column are significantly different ($p>0.05$)

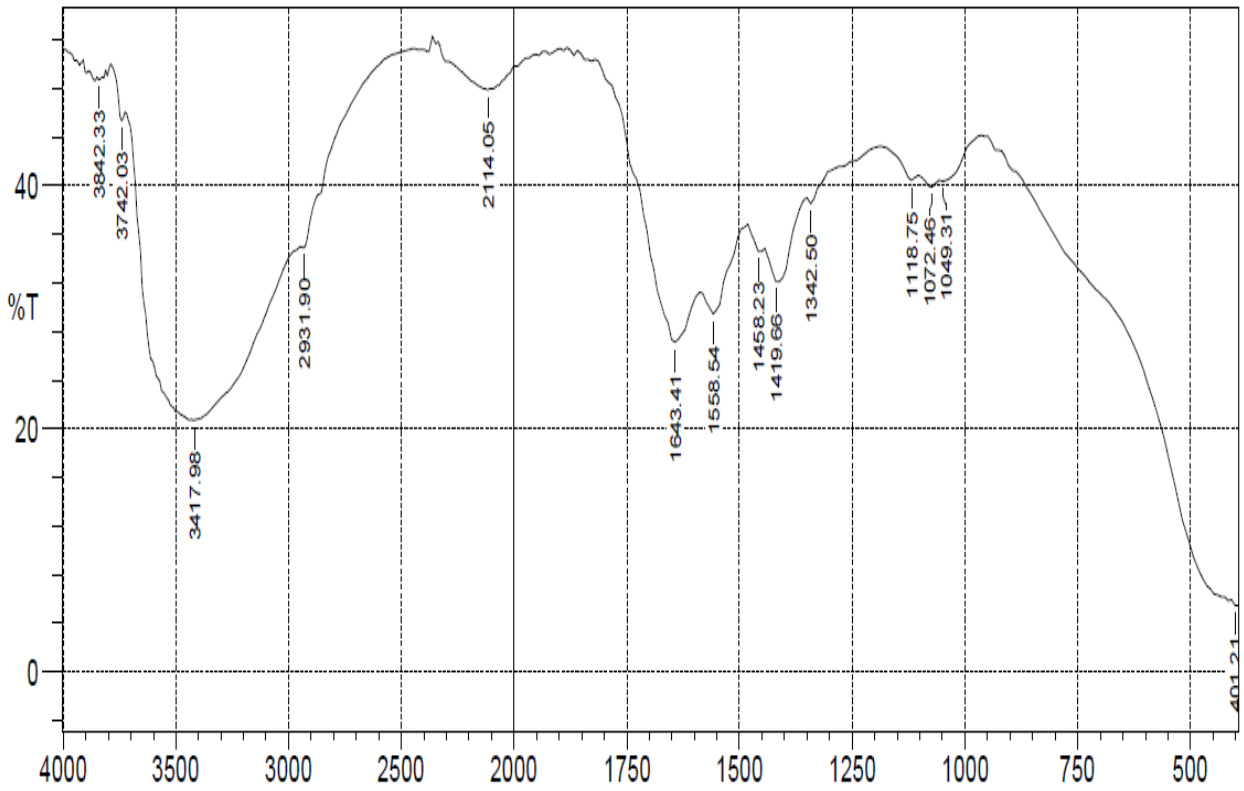
Appendix 2

Serum biochemical changes of hyperlipidemic and non- hyperlipidemic rats administered aqueous leaves extract and fraction 6 (F6) of *Tephrosia vogelii* and atorvastatinfor 21 days.

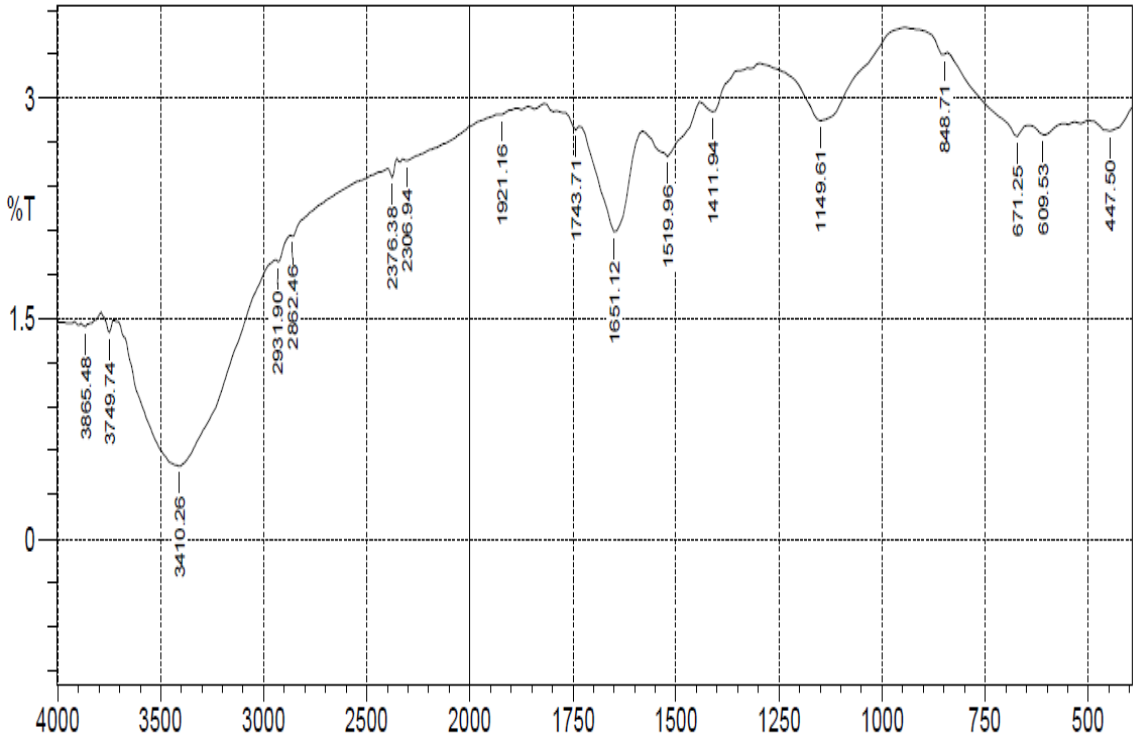
Groups	ALT (iu/L)	AST (iu/L)	ALP (iu/L)	Serum TB (iu/L)	Serum DB (iu/L)
NC	36.13± 1.43^{a}	173.00± 2.46^{c}	419.56±35.31^{c}	12.82±3.43^{a}	8.53± 1.43^{b}
HyperC	40.63± 4.20^{b}	181.00± 5.65^{d}	441.67± 45.92^{f}	18.07± 3.82^{c}	6.41± 3.26^{a}
Hyper+Aq100	39.93± 4.32^{a}	172.54± 6.12^{c}	432.25 ± 48.55^{e}	14.65± 5.24^{b}	9.76± 4.65^{c}
Hyper+Aq200	38.87± 7.45^{a}	171.43± 7.23^{c}	425.83± 33.45^{d}	14.74± 6.47^{b}	8.95± 5.46^{b}
Hyper+F(F6)100	37.57± 3.58^{a}	169.18± 6.26^{b}	414.25 ± 54.98^{b}	14.45± 7.33^{b}	9.88± 3.45^{c}
Hyper+F(F6)200	37.97± 4.23^{a}	159.98± 7.13^{a}	408.53± 24.45^{a}	14.25± 9.31^{b}	10.01±7.12^{d}
Hyper+Std	35.13± 2.46^{a}	165.00± 8.00^{b}	411.50± 39.73^{b}	14.12± 2.94^{b}	9.90± 3.7^{c}

Values are means ± SD of four determinations. Values with different superscripts down the column are significantly different ($p>0.05$).

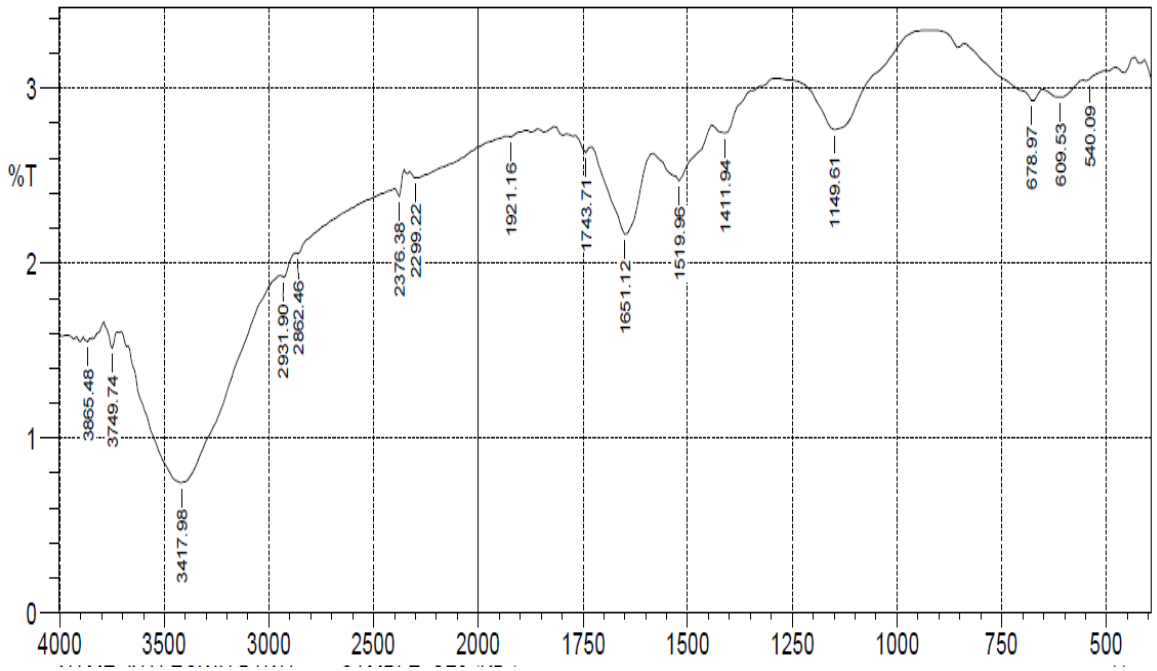
Appendix 3


%T
40
20
0
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
3842.33
3742.03
3417.98
2931.90
2114.05
1643.41
1558.54
1458.23
1419.66
1342.50
1118.75
1072.46
1049.31
401.21

Appendix 4


%T
3
1.5
0
3865.48
3749.74
3410.26
2931.90
2862.46
2376.38
2308.94
1921.16
1743.71
1651.12
1519.96
1411.94
1149.61
848.71
671.25
609.53
447.50
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500

Appendix 5


%T
3
2
1
0
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
3865.48
3749.74
3417.98
2931.90
2862.46
2376.38
2299.22
1921.16
1743.71
1651.12
1519.96
1411.94
1149.61
678.97
609.53
540.09

Appendix 6

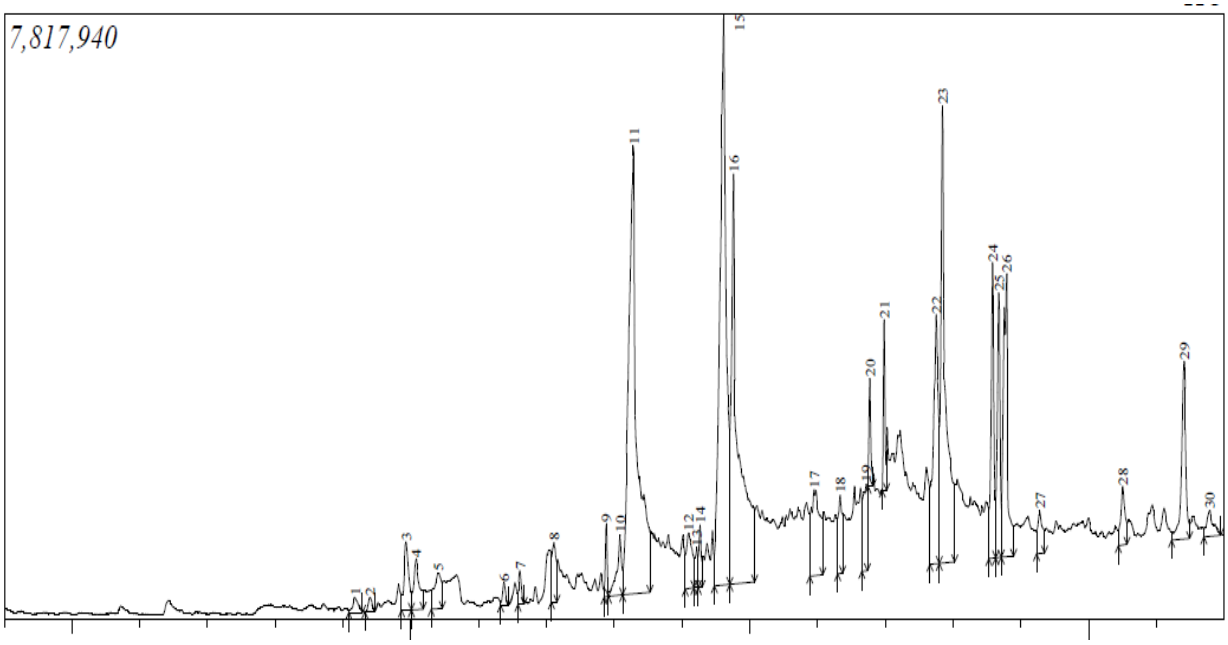


Figure 4.6: GC-MS spectrum for the bioactive fraction 6 of *Tephrosia vogelii*

Appendix 7

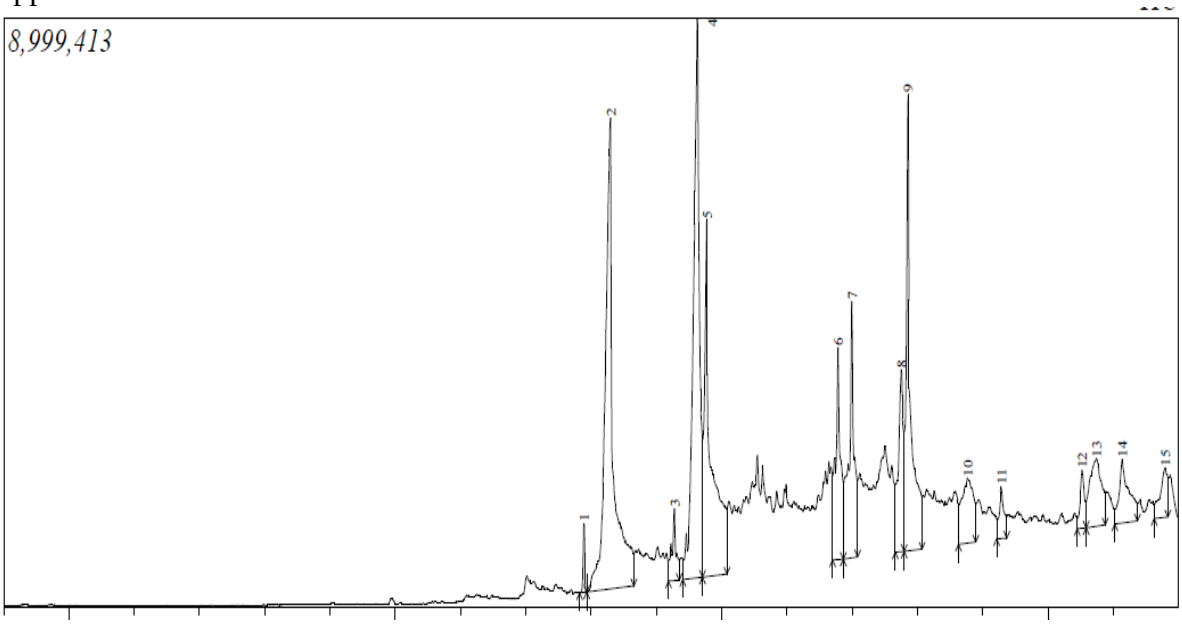


Figure 4.7: GC-MS spectrum for the bioactive fraction with Rf value 0.22 from TLC of *Tephrosia vogelii*

Appendix 8

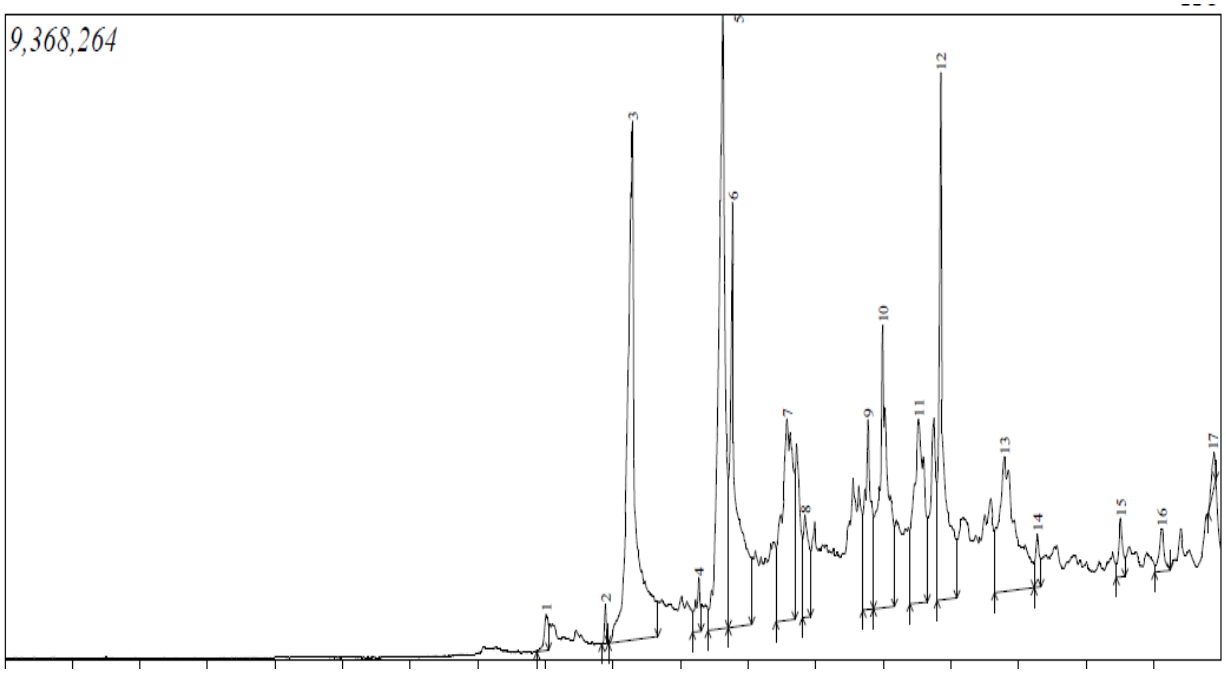


Figure 4.8: GC-MS spectrum for the bioactive fraction with Rf value 0.60 from TLC of *Tephrosia vogelii*