EVALUATION OF ANTI-HYPERLIPIDEMIC POTENTIAL OF ETHANOLIC LEAF EXTRACT OF *CLERODENDRUM VOLUBILE* P. BEAUV.

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ABSTRACT

This study investigated the effect of ethanolic leaf extract of *Clerodendrum volubile* on lipid profile of hyperlipidemic Wistar rats. The extract was screened and quantified for phytoconstituents according to standard methods. Also, acute and sub-acute toxicity of the extract were carried out on Wistar rats using standard methods. The rats were grouped as follows: General control (group 1) was given distilled water; hyperlipidemic control (group 2), injected intraperitoneally with Poloxamer-407; group 3 rats were administered Atorvastatin (70 mg/kg bwt) orally for 26 days before induction of hyperlipidemia while rats in phytopreventive group (groups 4 and 5) and curative group (groups 7 and 8) were administered sub-lethal doses of 250 and 500 mg/kg body weight orally. Hyperlipidemia was induced by single intraperitoneal injection of Poloxamer-407 (1.0 g/kg bwt) in rats within phytopreventive group two hours after 26 days of extract administration while rats in curative group were induced first with Poloxamer-407 two hours on 26th day (feeding with normal diet) before the administration of the extract for two days. All the animals were sacrificed on the 29th day and blood sample was separately collected through cardiac puncture. Antilipidemic activity was measured in the plasma of the rats by estimating the levels of lipidemic markers such as total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol, and triglycerides to determine the preventive effect and curative effect of the extract. The result revealed that *C. volubile* ethanolic leaf extract showed the presence of alkaloids, saponins, tannins, flavonoids, steroids and cardiac glycosides. Quantitative analyses of these phytochemicals showed a high concentration of phenolic, followed by flavonoids and alkaloids. A low concentration of saponin was observed while tannin content was the lowest. It was also observed that the levels of total cholesterol, LDL, VLDL cholesterol and triglycerides were significantly lowered in dose dependent manner in both phytopreventive and curative animals, administered 250 and 500 mg/kg body weight, then those animals in the hyperlipidemic control group while HDL level was significantly increased in dose dependent manner. Anti-hyperlipidemic activity was more efficient in *C. volubile* treated groups as compared to Atorvastatin (standard drug) treated animals. It was evident from this study that *C. volubile* leaf contained bioactive principles with hypolipidemic effect which were effective as curative agents than prophylactic agents.

Keywords: *Clerodendrum volubile*, phytochemicals, cardiovascular diseases, plasma lipids, Antihyperlipidemic.

INTRODUCTION

Cardiovascular disease (CVD), which include coronary heart disease (heart attacks), cerebrovascular disease, raised blood pressure (hypertension), peripheral artery disease, rheumatic heart disease, congenital heart disease and myocardial infarction (heart failure), are diseases involving the heart and blood vessels and research has been reported enormously that elevation of lipids in the blood are the risk factors for the development of cardiovascular diseases (Nelson 1996). Hyperlipidemia, an elevated level of blood cholesterol, triglyceride or both, was caused by hereditary factors. More commonly it is an acquired condition and constitutes major risk factor for cardiovascular disease (Nelson, 1996). Hyperlipidemia associated with lipid disorders are considered to cause atherosclerosis or hardening of the arteries (Saravanan *et al.,* 2003). Atherosclerosis is a condition that usually developed whenever lipids or fats substances called the plague builds up in the wall of arteries and thereby become narrower. This makes it difficult for blood to flow through and increase the risk of heart disease, stroke and other vascular diseases (Saravanan *et al.,* 2003).

Studies have shown that drugs synthesized and derived from herbal medicines contained bioactive principles that are capable of reducing abnormally increased in blood cholesterol or lipids. Examples of these include extracts of *Peritrophe bicalyculata* (Mansurah, 2011), *Moringa oleifera* (Chitresh *et al.,* 2012), *Gynostemma*

pentaphyllum (Megalli *et al.,* 2005) and *Indigo tinctoria* (Narender *et al.,* 2006). In addition, inclusion of leafy vegetables in diets had been reported to protect against chronic, degenerative and age-related diseases occurrence, due to the presence of antioxidants (Szeto *et al.,* 2002). Antioxidant therapy had been implicated in inhibition of atherosclerosis which results in prevention of the clinical complications of the disease such as cardiovascular diseases (Wilcox *et al.,* 1998).

Clerodendrum volubile belongs to the family *Lamiaceae* (*Verbenaceae*). It is a climbing shrub of about 3 m high with numerous greenish-white flowers (1½cm long). It is found in the deciduous forest and secondary jungle, across the region spanning Senegal to Fernando Po (Burkhill, 1985). The macroscopic study showed that *C. volubile* has a simple leaf with a reticulate venation, a cuminate apex and a cureate base. The leaves are green with a blond odour (Fred-Jaiyesimi and Adekoya, 2012). Ethnomedicinally, *C. volubile* leaves have been reported to be used as anti-aborifacients, analgestics, in general body healing, sedatives and also in the treatment of arthritis, rheumatism, dropsy and swellings (Erukainure *et al.*, 2010, Burkhill, 1985). In Nigeria, both the dried and blended fresh leaves are used as species in cooking (Erukainure *et al.,* 2011).

In search of less expensive alternative anti-hyperlipidemia drugs with minimal or no side effect, this study evaluates the anti-lipidemic potential of *C. volubile* ethanolic leaf extract on plasma lipid profile level in rats.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *C. volubile* were collected from Fashina Village, Ife-Central Local Government, Ile-Ife, Osun State. The plant material was identified and authenticated at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The specimen sample was deposited at IFE herbarium with specimen identification number IFE 7376.

Reagents and Chemicals

All the reagents used were of the analytical grade and were obtained from various sources such as British Drug House (BDH) Chemicals Limited; London, Sigma Chemicals Limited, St. Louis, Mo., U.S.A.; Diagnostic Chemical Kits from Randox Laboratories Ltd, United Kingdom and Atorvastatin (Pfizer Ireland Pharmaceutical, Ireland) was purchase from Campus Pharmacy, Obafemi Awolowo University, Ile-Ife. All solutions, buffers and reagents were prepared with distilled water and stored in the refrigerator at 4°C.

Experimental Animals

Forty (40) healthy Wistar rats of both sexes, of average weight 176 ± 19.09 g were obtained from the Animal House of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals were acclimatized for two weeks, fed with standard rat chow (Ladokun Feeds, Ibadan, Oyo State, Nigeria) and watered *ad libitum*. The animals were kept in a room maintained under environmentally controlled conditions of 24±1 °C and 12-h light: 12-h dark cycle.

Preparation of Ethanolic Extract

The leaves of *C. voluble* plant were harvested and air-dried for 72 hours. The dried leaves were milled into powdered form using manual grinder. Powdered plant sample (500 g) was suspended in 3 L of 70% (v/v) ethanol for 48 h at room temperature. The suspension was filtered through a double layer cheese-cloth and centrifuged at 3000 rpm for 10 minutes. The extraction process was repeated four times. The supernatant was combined and concentrated under reduced pressure on Edwards High Vacuum Pump Rotatory Evaporator (Edward Vacuum Co-operation, Crawley, England) at 35 ºC to give dark green residue. The dark green ethanolic extract was stored in a desiccator until required for further processing.

Phytochemical Screening

Phytochemical screening of the ethanolic extract of *C. volubile* was carried out based on standard procedures as described by Oyedapo *et al.* (1999), Trease and Evans (2002) and Sofowora (2006) for the presence of alkaloids, flavonoids, tannins, saponins, triterpenoids, cardiac glycosides and steroids.

Estimation of Total Flavonoids.

The concentration of flavonoids in the extract was estimated spectrophotometrically according

to the procedure of Sun *et al.* (1999). The extract (0.1 g) was dissolved in 20 ml of 70 % (v/v) ethanol. To each of the clean dry test tubes (in triplicate) was pipetted 0.5 ml of working solution of sample and diluted with 4.5 ml distilled water. In addition, the following were added to each of the test tubes 0.3 ml of 5 % (w/v) $NaNO_2$, 0.3 ml of 10 % $AlCl_3$ and 4 ml of 4 % (w/v) NaOH. The reaction mixture was incubated at room temperature for 15 minutes, the absorbencies' of the products were read at 500 nm against reagent blank. The standard curves of the estimation of total flavonoid concentration were prepared by separately pipetting 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 ml of 1 mg/ml rutin into clean dry test tubes. Each of these volumes was diluted into 5 ml with distilled water. To each of the tubes were added 0.3 ml of 5 % (w/v) $NaNO_2$, 0.3 ml of 5 % (w/v) $AlCl_3$ and 4 ml of 4 % (w/v) NaOH. The reaction mixtures were incubated at room temperature for 15 minutes. Absorbance was taken at 500 nm and was plotted against the concentration to give the standard curve.

Estimation of Total Phenolics

Estimation of total phenolics concentrations was carried out using Folin-Ciocalteu's reaction reported by Singleton *et al.,* (1999). The assay involved pipetting 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 ml of garlic acid solution (1.0 mg/l) in triplicate. Each volume was made up to 1.0 ml with distilled water. To each of the test tubes was added 1.5 ml of 10 % (w/v) $NaHCO_3$ solution to give a total volume of 4.0 ml. The reaction mixtures were further incubated for additional one and half hours. The estimation of phenols in the ethanolic extract involved pipetting 0.5 ml each of 5 mg/ml ethanolic extract into clean dry test tubes in triplicate. The volumes were adjusted to 1.0 ml with distilled water. To each of the tubes was added 1.5 ml of Folin-Ciocalteu's phenol reagent (1:10). The reaction mixture was incubated at room temperature for 5 minutes. Thereafter, 1.5 ml of 10 % (w/v) $NaHCO_3$ solution was added to each of the reaction mixtures and incubated for one and half hour (1½ h). The absorbance was read at 725 nm against the control containing all reagents except the standard gallic acid. The absorbance at 725 nm was plotted against the concentration to produce the standard curve.

Estimation of Alkaloid Content

Estimation of alkaloid content was carried out as previously described (Kam *et al.*, 1999; Yang *et al.*, 1999). Extract (40 g) was dissolved in 200 ml of 5% (v/v) HCl solution followed by successive partitioning of acidic filtrate with benzene (l00 ml x 3), chloroform (100 ml x 3) and ethyl acetate (100 ml x 3) to remove non-alkaloid materials. The acidic aqueous solution was basified with ammonia solution to pH 12, followed by the extraction of released alkaloids with chloroform (150 ml x 5). The chloroform extracts were combined followed by evaporation at 35 °C to dryness under reduced pressure and weighed.

Estimation of Saponin Content

Estimation of saponin content was carried out as previously described by Abdel-Gawad *et al.* (1999) and Wagner *et al.* (1984). The ethanolic extract (40 g) was washed twice with chloroform (50 ml x 2) and, then twice with ethyl acetate (50ml x 2). The residue was dissolved in 20% (v/v) ethanol and the solution was extracted three times (100 ml x 3) with butanol, on evaporation, the obtained residue was taken up in methanol. The residue was dissolved in 50 ml 50% (v/v) methanol followed by the addition of diethyl ether (100 ml) to precipitate crude saponins. The upper diethyl ether layer was carefully removed; the residue was dissolved in a little amount (10 ml) of methanol and was poured into diethyl ether (200 ml). The upper diethyl ether was again removed and the residue was dissolved as described earlier and precipitated by the addition of diethyl ether. The precipitate was dried in the oven to a constant weight.

Estimation of Tannins Content

Estimation of total tannins content was carried out according to the method of Van-Burden and Robinson (1981) using tannic acid (0.02 mg/ml) as a standard. The reaction mixture consisted of 0.2 ml of the extract (1 mg/ml stock), 0.2 ml ammonium ferric citrate (3.5 g/l), and 0.2 ml of 20% (v/v) ammonia. The reaction mixture was incubated at room temperature for 10 min. after which the absorbance was read at 525 nm, against the reagent blank on a Spectrophotometer (S23A, ESCHMED Medical, England). The assay was carried out in triplicates and the mean of the three readings was obtained. The standard (tannic acid) calibration curve was prepared by separately

pipetting 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml tannic acid solution (0.02 mg/ml) in triplicates into clean test tubes. The volumes were made up to 2.0 ml with distilled. The concentration of tannins in the extract was extrapolated from standard calibration curve and expressed as milligram tannic acid equivalent per g of extract (mg TAE/g extract).

Acute Toxicity Study

Acute toxicity study was carried out according to the modified procedure of Lorke's (1983) in two phases. In this study, eighteen (18) healthy Wistar albino rats of both sexes with average weight of 79 ± 8.58 g were randomly divided into six groups of 3 rats in each cage. In phase one, nine (9) rats randomly divided into three groups were administered single dose of 10, 100 and 1000 mg/kg bwt of the ethanolic extract of *C. volubile* respectively. Changes in general behavioural pattern and mortality of rats were monitored for 24 h.

The second phase of the test was performed after 24 h based on the observation and number of survivors noted in first test. Another set of nine (9) rats were randomly divided into three groups and were administered (orally) 1600, 2900 and 5000 mg/kg bwt respectively. The rats were observed for toxic symptoms at 30 min, 1, 2, 6 and 24 h after test substance administration. The visual changes, behavioural pattern and mortality of rats were monitored for 48 h. The number of survivors was noted after 48 h for each animal. The LD_{50} was estimated from the plot of percentage mortality versus logarithm of concentrations.

Grouping and Treatment of Experimental Animals

Forty (40) Wistar rats were divided into 8 groups of 5 animals per group. The experimental groups are as shown below:

Group1: Rats served as control and received distilled water;

Group 2: Rats were injected 1.0 g/kg bwt Poloxamer-407 intraperitoneally 48 h (on the 26th day) prior to blood collection;

Group 3: Rats were administered Atorvastatin 70 mg/kg bwt for 26 days before induction of hyperlipidemia by injection of 1.0 g/kg bwt Poloxamer-407 intraperitoneally;

Group 4: Rats were administered 250 mg/kg bwt ethanolic extract for 26 days before induction of hyperlipidemia;

Group 5: Rats were administered 500 mg/kg bwt ethanolic extract for 26 days before induction of hyperlipidemia;

Group 6: Rats were fed normally with rat pellet and on the 26th day, hyperlipidemia was induced.

They were allowed for two hours before oral administration of Atorvastatin (70 mg/kg kg bwt) for two days;

Group 7: Rats were fed normally with rat pellet and on the 26th day, hyperlipidemia was induced. They were allowed for two hours before oral administration of 250 mg/kg bwt ethanolic extract for two days.

Group 8: Rats were fed normally with rat pellet and on the 26th day, hyperlipidemia was induced. They were allowed for two hours before oral administration of 500 mg/kg bwt ethanolic extract for two days.

Preparation of Standard Drug

Atorvastatin (Pfizer Ireland Pharmaceuticals, Ireland) (anti-hyperlipidemic drug) tablets (20 mg) were used as a reference drug and were purchased from Campus Pharmacy of Obafemi Awolowo University, Ile- Ife, Nigeria. The tablets were crushed into powder, weighed, dissolved in distilled water.

Induction of Hyperlipidemia

Hyperlipidemia was induced according to the method described by Megalli *et al.* (2005). Typically, Poloxamer-407(1.0 g/kg bwt) was introduced to the animals intraperitoneally. The syringes were placed in ice prior to Poloxamer-407 administration to maintain the polymer in a mobile viscous state during the injection, since Poloxamer-407solutions at concentration greater than about 23% (w/w) exhibit reverse thermal gelatin properties.

Sacrificing Experimental Animals

On the 29th day, the animals were sacrificed under slight chloroform anaesthesia. The blood was

collected by cardiac puncture into heparinised bottles for plasma preparation.

Preparation of Blood Plasma

The blood sample collected in heparinised bottles was centrifuged at 3000 rpm for 10 min. The supernatant (plasma) was collected, stored in sterile vials and kept in the freezer at 0 °C for biochemical analyses.

Biochemical Assays

The plasma samples were analyzed for total cholesterol, low density lipoprotein (LDL) -cholesterol, high density lipoprotein (HDL) -cholesterol triglycerides and very low density lipoprotein (VLDL) -cholesterol according to the procedure described in the sigma cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides assay kits (Randox laboratory Ltd, United Kingdom).

Estimation of Plasma VLDL-Cholesterol Concentration

The plasma VLDL-cholesterol was determined according to the method of Friedwald *et al* (1972) using the expression below.

VLDL cholesterol =

The concentration of VLDL-c in the plasma was expressed as mg/dl.

Estimation of Plasma LDL Cholesterol Concentration

Plasma LDL-cholesterol was determined according to the method described by Sundkamp *et al.* (1990) using the equation below:

LDL-cholesterol = Total plasma cholesterol – (HDL-c +VLDL-c).

The concentration of LDL-c in the plasma was expressed as mg/dl.

STATISTICAL ANALYSIS

Data were expressed as mean ± standard error of mean (SEM). The significance of the results was evaluated using analysis of variance (ANOVA) and the means were compared using Turkeys test. Values of $p < 0.05$ were regarded as statistically significant.

RESULTS

Phytochemical Constituents of Ethanolic Extract of *C. volubile*

The summary of the phytoconstituents in the ethanolic leaf extract of *C. volubile* is as shown in Table 1. The extract gave positive reaction tests for the presence of alkaloids, flavonoids, tannins, triterpenoids, saponins, steroids and cardiac glycosides.

Concentrations of identied phytochemicals in Ethanolic Leaf Extract of *C. volubile*

Concentrations of tannin, phenolic, flavonoid, alkaloid and saponin in the ethanolic leaf extract of *C. volubile* are summarised in Table 2. Alkaloids had the highest concentration, followed by flavonoids and phenols respectively while saponin and tannin were at very low concentrations.

Acute Toxicity of Crude Ethanolic Leaf Extract of *C. volubile* **in Wistar Rats**

No death was recorded in the first and second phase of the test after oral administration but signs of weakness and loss of appetite in animals treated with a single dose of 10 – 1000 mg/kg body weight in phase 1 and 1,600 – 5000 mg/kg body in phase 2 of the study were observed. The median lethal dose (LD_{50}) of *C. volubile* ethanolic leaf extract in the experimental rats was estimated to be greater than 5000 mg/kg body.

Effects of Ethanolic Leaf Extract of *C. volubile* **on Plasma Lipid Profiles of Wistar Rats**

The summary of the effects of the ethanolic leaf extract of *C. volubile* on plasma lipid profiles of Poloxamer-407- induced hyperlipidemia rats are shown in Table 3. In general, the total cholesterol, low-density lipoprotein-cholesterol (LDL-c), triglycerides (TG) and very low-density lipoprotein-cholesterol (VLDL-c) level in rats within the normal control (group 1) was significantly ($p<0.05$) lower than that of all the other groups. The level in hyperlipidemic control rats (group 2) was significantly ($p<0.05$) higher than all the other groups. Total cholesterol level was significantly lower in the Atorvastatin-treated group when compared with Poloxamer-407-treated group. The two doses of the extract (250 and 500 mg/kg body weight) in both pre-treated (groups 4 and 5)

and post-treated (group 7 and group 8) rats showed a dose-dependent protective effect. The high density lipoprotein cholesterol (HDL-c) level of rats given Atorvastatin (groups 3 and 6) and doses of the *C. volubile* ethanolic leaf extract (250 and 500 mg/kg body weight; groups 7 and 8) increased significantly ($p < 0.05$) compared to the hyperlipidemic control rats. The low-density lipoprotein-cholesterol (LDL-c) and triglyceride level of rats given Atorvastatin in both pre-treated (group 3) and post-treated (group 6) groups reduced significantly ($p < 0.05$) compared to the hyperlipidemic control (group 2) in a dose-dependent manner. Likewise, same trend was observed in LDL-c and triglyceride levels in the animal pre-treated (groups 4 and 5) and post-treated (groups 7 and 8) with 250 and 500 mg of the extract.

Table1: Phytochemical constituents of Ethanolic Leaf Extract of *C. volubile*

Phytochemical	Results
Alkaloids	+
Flavonoids	+
Tannins	+
Triterpenoids	+
Saponins	+
Steroids	+
Cardiac glycoside	+

(+) represent presence of the phytochemical

Table 2: Concentrations of identified Phytochemical Constituents in the Ethanolic Leaf Extract of *C. volubile*

	Weight of Extract (g)	Metabolite Concentration (g)
Total flavonoid (mg/g RE/ g extract)	0.1	$0.302 \pm 6 \times 10^{-4}$
Total Phenolic (mg/g GAE/ g extract)	0.025	$0.103 \pm 6 \times 10^{-4}$
Tannins (mg/g TAE/ g extract)	20	$0.037 \pm 1 \times 10^{-4}$
Alkaloid (g)	40	$0.320 \pm 6 \times 10^{-3}$
Saponin (g)	40	$0.080 \pm 6 \times 10^{-4}$

Each value represented the mean ± SD, n = 3 determinations.
TAE (Tannic Acid Equivalent)
GAE (Gallic Acid Equivalent)
RE (Rutin Equivalent

Table 3: Effects of Ethanolic Leaf Extract of *C. volubile* on Plasma Lipid Profile of Wistar Rats

Group	Parameter				
	T C (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	TG (mg/dl)	VLDL-c (mg/dl)
Group 1 (control)	187.56±2.66	83.92±14.30	87.32±1.86	81.60±7.75	16.32±1.55
Group 2 (Hyperlipidemic control)	465.96±9.39[a]	19.45±2.31[a]	303.08±13.4[a]	717.14±119[a]	143.43±23.96[a]
Group 3 (70 mg/kg bwt) (Atorvastatin) (pre-treatment)	352.95±8.16[b] ↓	79.34±6.25[b] ↑	225.34±7.22[b] ↓	557.52±12.5[b] ↓	48.27±4.63[b] ↓
Group 4 (250 mg/kg bwt) (pre-treatment)	359.90±8.57[b] ↓	77.49±2.95[b] ↑	236.05±5.04[b] ↓	231.86±32.7[b] ↓	46.37±7.30[b] ↓
Group 5 (500 mg/kg bwt) (pre-treatment)	213.32±2.43[b] ↓	89.54±0.72[b] ↑	91.02±1.75[b] ↓	163.79±21.1[b] ↓	32.76±3.15[b] ↓
Group 6 (70 mg/kg bwt) (Atorvastatin) (post-treatment)	374.75±8.64[b] ↓	131.98±13.5[b] ↑	182.94±7.28[b] ↓	299.28±18.16[b] ↓	59.82±3.60[b] ↓
Group 7 (250 mg/kg bwt) (post-treatment)	362.30±2.94[b] ↓	165.20±4.39[b] ↑	150.94±3.56[b] ↓	230.79±37.7[b] ↓	46.15± 7.54 [b] ↓
Group 8 (500 mg/kg bwt) (post-treatment)	264.76±5.12[b] ↓	185.45±10.54[b] ↑	44.83±6.71[b] ↓	172.41±16.00[b] ↓	34.48±3.20[b] ↓

Values are expressed as mean ± SEM, n = 5 replicates. Values with ([a]) are statistically significant at $p < 0.05$ compared to the control (group 1), while values with ([b]) are statistically significant at $p < 0.05$ compared to the hyperlipidemic control (group 2). TC represents (Total cholesterol), HDL-c (High-density lipoprotein-cholesterol), LDL – c (Low-density lipoprotein-cholesterol), TG (Trglycerides) and VLDL-c (very low-density lipoprotein-cholesterol). ↓ represents decrease in concentration, ↑ represents increase in concentration.

DISCUSSION

The phytochemical are physiologically active compounds exhibiting and possessing great potential for therapeutic uses. It is important to note that medicinal plants contain mixtures of different chemical compounds that act individually, or synergistically to improve health (Gurib-Fakim, 2006). Phytochemicals such as tannins and phytates are regarded as botanical chelators and possess potential malaria suppressive effects through sequestration of iron (Etkins, 1996). Moreover, saponins, polyphenols and phytosterols exhibit hypocholestrolemic effects on animals and humans thereby contributing to the phenomenon of the low rate (incidence) of atherosclerosis (Johns and Chapman, 1995; Johns, 1996). Flavonoids on the other hand are actively involved in plant metabolic processes especially energy transfer, control of respiration, photosynthesis, action of growth hormones and growth regulations as well as morphogenesis, sex determination and defense against infections (Middleton and Kandaswami, 1994; Middleton, 1996). Phenols are very important phytochemicals because of their ability to scavenge free radicals owing to their redox properties which permit them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Rice – Evans *et al.,* 1997).

In this study, phytochemical screening of ethanolic leaf extract of *C. volubile* revealed the presence of flavonoids, alkaloids, saponins, tannins, triterpenes, cardiac glycosides and steroid. The quantitative estimation of the extract showed that it contained high concentration of phenolics, this was followed by flavonoids and alkaloids. A low concentration of saponins and tannins was observed. The high polyphenol content of the *C. volubile* leaf is likely to be responsible for its various therapeutic properties such as antioxidant activities (Kris-Etherton *et al.*, 2002; Vaya *et al.*, 2003), hypolipidemic potential

(Narender *et al*., 2006; Harnafi and Amrani, 2007) and vaso-relaxant activities (Bernatova *et al*., 2002).

Saponin is known to possess serum cholesterol lowering activity (Topping *et al.,* 1980). The cholesterol lowering ability of saponin is based on the fact that it binds either bile acids or cholesterol in the intestinal lumen. Binding with cholesterol will make it more easily re-absorbed causing a reduction in enterohepatic circulation of bile acid in the liver (Potter *et al.,* 1979; Kritchevoky, 1997).

Calcium in the form of calcium carbonate and calcium lactate are also believed to be capable of binding bile acid in the intestinal lumen suggesting that it may be involved in the bile acid binding and cholesterol lowering in a manner similar to saponin (Marke, 1991). The *de novo* synthesis of calcium lactate may be enhanced by components of the extract, thus contributing to the cholesterol lowering property mediated with this extract.

Changes in the animals' major lipids profile such as cholesterol, high and low-density lipoprotein cholesterols and triglycerides provides information on metabolism of lipids and predisposition of animals to cardiovascular risk (Yakubu *et al*., 2008).

The higher triglyceride levels compared to other lipids have been attributed to the inhibition of triglyceride degradation which might be as a result of a direct inhibitory effect on lipoprotein lipase bound to capillary endothelium. Lipoprotein lipase is vital in the metabolism of triglycerides and is involved in several pathological disorders, including atherosclerosis and obesity (Wassan *et al*., 2003; Megalli *et al*., 2005). Hence, it is possible that the ethanolic leaf extract of *C. volubile* affects lipoprotein lipase activity because the extract was significantly effective in reducing triglycerides levels in both pre-treated (group 4 and 5) and post-treated (group 7 and 8) rats compared to rats in hyperlipidemic control (group 2).

Atorvastatin, belongs to a class of drug called statins, are used in lowering cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver. Increased cholesterol levels have been associated with cardiovascular diseases (CVD), and statins are therefore used in the prevention of these diseases (Ray *et al.,* 2001). Atorvastatin acts by competitively inhibiting HMG-CoA reductase, the enzyme that is involved in the first committed step of cholesterol biosynthesis. However, since all members of the class of statins are similar to HMG-CoA on a molecular level, Atorvastatin substitutes HMG-CoA in the enzyme and reduce the rate by which it is able to synthesize mevalonate, which is required for the biosynthesis of cholesterol (Ray *et al.,* 2001).

The rats pre-treated (group 3) and post-treated (group 6) with Atorvastatin (70 mg/kg bwt) showed significant reduction in triglycerides level by 22.26% and 58.27% respectively compared to hyperlipidemic control (group 2). It was observed that ethanolic extract of *C. volubile* was able to reduce triglycerides levels significantly in a dose-dependent manner in the pre-treated (groups 4 and 5) rats at 250 mg/kg and 500 mg/kg bwt by 67.67% and 77.16% respectively compared to hyperlipidemic control (group 2). Also, in post-treated (groups 7 and 8) rats, triglycerides levels reduced significantly in a dose-dependent manner at 250 and 500 mg/kg bwt of the extract by 67.82% and 75.96% respectively when compared to group 2 (hyperlipidemic control). The significant reduction of plasma triglycerides by *C. volubile* extract could be attributed to the presence of bioactive principles. Examples of antilipidemic and antioxidant compounds include ellagic acid (tannin), sanguinarine (alkaloid), saponins (sinigrin and sinalbin) and narigin (flavonoids) (Nijveldt *et al.,* 2001; Rathbone and Bruce, 2002).

The plasma total cholesterol level of Poloxamer-407 treated rats (group 2) increased significantly by 58.95% when compared to that of normal control (group 1) rats. Administration of 250 mg/kg bwt and 500 mg/kg bwt doses of the ethanolic extract after intraperitoneal injection of Poloxamer-407 (1.0 g/kg bwt) resulted in significant decrease in plasma cholesterol levels in pre-treated (groups 4 and 5) rats by 21.24% and 53.32% respectively and post-treated (groups 7 and 8) rats by 21.72% and 44.05% respectively. The total cholesterol levels of the rats pre- treated with Atorvastatin (70 mg/kg bwt) in group 3 and

ethanolic extract (250 and 500 mg/kg bwt) in groups 4 and 5 was reduced by 22.76%, 21.24% and 53.32% respectively, while the total cholesterol levels of rats post-treated with Atorvastatin (70 mg/kg bwt) in group 6 and ethanolic extract (250 mg/kg and 500 mg/kg bwt) in groups 7 and 8 was decreased by 17.99%, 21.72% and 44.05% respectively. Significant reduction in plasma cholesterol ($p< 0.05$) in both pre-treated (groups 3, 4 and 5) and post-treated (groups 6, 7 and 8) rats could be attributed to presence of flavonoids and saponins in the plant extract. Flavonoid has been proved to inhibit cholesterol synthesis, cellular cholesterol esterification and 3-hydroxyl-3-methylglutaryl Coenzyme A reductase (Anderson *et al.*, 1995). It was reported that the intake of protein-rich in isoflavonoids (50 g) significantly reduced serum cholesterol level (Vladimir and Daniela, 2005). The citrus flavonoids (naringenin and hesperetin) had been reported to decreased cholesterol synthesis by inhibiting acyl CoA cholesterol acyl transferase (ASAT) activity in Hep G_2 cells (Wilcox *et al.*, 1998).

In Atorvastatin pre-treated rats (group 3), there was a significant increase in HDL-cholesterol level by 75.49% compared to hyperlipidemic control (group 2) but in rats (groups 4 and 5) pre-treated with extract at 250 mg/kg and 500 mg/kg bwt respectively, there was no significant difference ($p> 0.05$) compared to group 1 (control). In groups 4 and 5, HDL–cholesterol level showed a dose dependent increase by 74.90% and 78.28 % respectively.

Moreover, rats (group 6) post-treated with Atorvastatin (70 mg/kg bwt) showed a significant increase in HDL-cholesterol level by 85.26% compared to group 2 (hyperlipidemic control) while groups 7 and 8 rats post-treated with extract (250 and 500 mg/kg bwt) showed a dose dependent significant increase in HDL-cholesterol levels by 88.23% and 89.51% respectively compared to group 2. While facilitation of atherogenesis by LDL-cholesterol was due to its role in depositing cholesterol in vascular bed, HDL-cholesterol carries out the reverse transport of excess cholesterol deposited in vascular bed by LDL from cells of tissues to the liver. The increased levels of plasma HDL-cholesterol concentration in rats (groups 4 and 5) pre-treated with ethanolic extract at 250 and 500 mg/kg bwt respectively and rats (groups 7 and 8) post-treated with ethanolic extract at 250 and 500 mg/kg bwt respectively suggest that *C. volubile* has the potential to prevent the formation of atherosclerosis and coronary heart disease, which are the secondary complications of hyperlipidemia (Narender *et al.*, 2006).

Considering the effect of *C. volubile* on plasma LDL-cholesterol levels, intraperitoneal injection of Polxamer-407 resulted in a two-fold significant rise in LDL-cholesterol by 55.76% in group 2 compared to normal control (group 1). In rats (group 3) pre-treated with Atorvastatin (70 mg/kg bwt), there was significant reduction in LDL–cholesterol by 55.76% compared to group 2 (hyperlipidemic control). Also, in rats (group 6) post-treated with Atorvastatin (70 mg/kg bwt), there was marked ($p< 0.005$) reduction of LDL-cholesterol level by 25.65% compared to hyperlipidemic control (group 2). Rats (group 4 and 5) pre-treated with 250 mg/kg and 500 mg/kg bwt of extract showed a dose-dependent significant reduction in LDL-cholesterol levels by 22.12% and 69.97% respectively compared to hyperlipidemic control (group 2). More so, rats (groups 7 and 8) post-treated with extract (250 and 500 mg/kg bwt) showed a dose dependent significant reduction in LDL-cholesterol levels by 88.23% and 89.51% respectively compared to hyperlipidemic control (group 2). Low- density lipoprotein-cholesterol (LDL-c) is primary carrier of plasma cholesterol and is referred to as bad cholesterol because it builds up slowly in the walls of arteries feeding the heart and brain. Consequently, it forms plaque that clots the arteries thereby causing atherosclerosis which may lead to stroke (Jackson, 1996; Yakubu *et al.*, 2003). Also, flavonoids have been reported to play a major role in reducing the risk of cardiovascular diseases by decreasing the blood plasma low density lipoprotein lipids (Narender *et al.,* 2006). It could be surmise that the ethanolic leaf extract of *C. volubile* possesed active principles such as flavonoids (Rastogi and Mehrotra, 1991), saponin (Wang *et al.,* 2013) and alkaloids (Chitresh *et al.,* 2012) that prevent atherosclerosis and cardiovascular diseases.

It was also observed that the VLDL-cholesterol levels increased by eight-fold in group 2 when Poloxamer-407 was injected rats compared to normal control rats (group 1). Atorvastatin treated rats (group 6); showed significant reduction in the VLDL-cholesterol by 58.29% compared to group 2 (hyperlipidemic control), while group 3 rats (pre-treated with atorvastatin) showed significant reduction in VLDL-cholesterol level by 66.35% compared to group 2 (hyperlipidemic control). Rats (groups 4 and 5) pre-treated with 250 and 500 mg/kg bwt extract showed a significant decrease in VLDL-cholesterol level in a dose-dependent manner by 71.60% and 75.77% respectively compared to group 2 (hyperlipidemic control), while rats (group 7 and 8) post-treated with 250 and 500 mg/kg bwt extract also showed similar trends in VLDL-cholesterol levels reduction by 67.82% and 75.96% respectively compared to group 2 (hyperlipidemic control).

The results of this study revealed that *C. volubile* ethanolic leaf extract was able to reduce the level of hyperlipidemia in both pre-treated (group 4 and 5) and post-treated (group 7 and 8) rats. The ethanolic leaf extract compare better with Atorvastatin (reference anti-lipidemia drug) in suppressing the effects of Poloxamer-407-induced hyperlipidemia in rats.

From the study it could be deduced that the use of *C. volubile* ethanolic leaf extract as a post-treated drug elicits a better therapeutic effect than its use as pre-treated drugs. This is because *C. volubile* lower LDL-cholesterol, total cholesterol; VLDL-cholesterol and triglycerides significantly in post-treated rats in a dose-dependent manner than pre-treated rats suggesting that the extract will be a better curative agent than a prophylactic agent.

Also, *C. volubile* extract showed a better therapeutic effect as a pre-treated drug than Atorvastatin (a standard anti-lipidemic drug) because the extract lowered LDL-cholesterol, total cholesterol, VLDL-cholesterol and triglycerides significantly in pre-treated rats in a dose-dependent manner than Atorvastatin-treated rats>suggesting that the extract will be a better alternative therapy in the treatment and management of hyperlipidemia and its attendant complication. Also, the HDL-cholesterol level was observed to increase significantly in a dose-dependent manner in both pre-treated and post-treated compared to the animals in the hyperlipidemic control.

Hyperlipidemia observed in this study particularly hypercholesterolemia could probably be as a result of enhanced fatty acid oxidation to acetyl-CoA and the excess acetyl-CoA so produced is channeled to cholesterol synthesis (West *et al.,* 1996).

CONCLUSION

It was evident from this study that *C. volubile* leaf contained phytochemicals with anti-hyperlipidemic activity which were more effective as curative agent than a prophylactic agent. Its high flavonoids and phenolic contents could be a plausible explanation for this activity. Hence, the plant could therefore be listed in management and treatment of hyperlipidemia-related diseases.

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