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Hypolipidemic Effect of Ethanol Extract of Bushbuck (*Gongronema latifolium* Benth.) Leaves on High- Fat Induced Hyperlipidemic Rats

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Abstract

This study was designed to investigate the in-vitro antioxidant activity and in-vivo hypolipidemic effect of ethanol extract of Gongronema latifolium leaves in hyperlipidemic rats. Fourteen (14) Wister albino rats were randomly divided into 7 groups of two Wister albino rats each. Twelve of the Wister rats were experimentally hyperlipidemic induced whereas 2 Wister rats served as normal control. Group C – F from the induced hyperlipidemic rats were treated with different concentrations of the extract (500mg/kg/body weight, 250mg/kg.b.w, 125mg/kg.b.w, and 62.5mg/kg.b.w and Group B was administered Simvastatin (a standard lipid lowering drug, 10mg/kg), Group H was fed with high fat diet and water alone. The aim of the study was to determine the in vitro antioxidant properties of G. latifolium in comparison to the standard drug Simvastatin. Antioxidant studies were conducted in-vitro and the DPPH radical scavenging, anti-lipid peroxidation and nitric oxide inhibition activity were calculated. The result showed an inhibitory effect of ethanol extract of G. latifolium leaves which decreased as the concentration of the extract decreases. The DPPH scavenging activity, anti lipid peroxidation and nitric oxide inhibition activity were 73.52±5.0, 59.13± 4.6, 53.99±5.0 respectively at 100 mg/ml

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concentration which were significant ($p<0.05$) when compared with the standard inhibition activity of 99.01±0.5, 99.41±0.1, 98.68±0.4 respectively at 100 mg/ml concentration and this inhibition was more for the DPPH scavenging activity. The result of the work shows the ameliorative effect of ethanol extract of G. latifolium leaves on hyperlipidemia as it significantly ($P\leq0.05$) reduced total cholesterol, triglyceride and LDL-c levels. LDL-c levels reduced from 60.8± 1.1 in the negative control to 28.7± 0.3 for 500 mg/kg concentration of extract and there was no significant difference at probability level of 0.05 in Simvastatin treated group (26.8±0.7) and 500 mg/kg.b.w concentration of extract (28.7± 0).The HDL-c level increased from 30.3±2.7 in the negative control to 43.1±1.5 in the 500 mg/kg extract concentration and 47.2±1.6 for the Simvastatin treated group. The significant increase of HDL-c and decrease of LDL-c shows the ameliorative effect of G. latifolium leaves which increases with an increase in concentration of the extract. The result of this study alluded that G. latifolium leaves has good antioxidant and hypolipidemic properties which can be of pharmacological importance because nutraceuticals or phytotherapeutics can be made out of it so that mankind may begin to effectively utilize the plant for maximal benefit.

Keywords: hyperlipidemia, hypolipidemic, antioxidant, *Gongronema latifolium,* Simvastatin, high fat diet.

Introduction

Lipids, at proper levels perform important functions in your body, but can cause health problems if in excess. Hyperlipidemia is caused by increased plasma lipid levels, mainly cholesterol (TC), triacylglycerols (TG), and low density lipoproteins (LDL) along with decrease in high density lipoprotein (HDL) and it is core in initiation and progression of cardiovascular diseases (Ghule *et al.,* 2006). Therefore, prime consideration in therapy for Hyperlipidemia is to enervate the elevated plasma levels of TC, TG, and LDL along with increase in HDL lipids (Ghule *et al.,* 2006). Hyperlipidemia is a risk factor for the development of cardiovascular diseases (CVD), including atherosclerosis (AS), coronary heart diseases (CHD) and hypertension.Oxidative stress initiated by free radicals is a major

contributor to CVD development. Scientists have developed increased interest in functional foods because of the serious side effects of cholesterol-lowering drugs.

Antioxidants comprises of a range of substances that play a role in protecting biological systems against the deleterious effects of oxidative processes on macromolecules such as proteins, lipids, carbohydrates. Many of these substances contribute to the prevention and treatment of diseases which reactive oxygen species produced during metabolism and other activities that is beyond the antioxidant capacity of a biological system. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Free radical damage may lead to cancer and cardiovascular diseases. Examples of antioxidants include beta-carotene, lycopene, vitamins C, E, A and others (Hamid *et al.*, 2010). An antioxidant is capable of slowing or preventing the oxidation of other molecules. There is an increased focus on plants in search for appropriate hypolipidemic agents; firstly because the traditional medicine practitioners reported that natural products may be better treatment than currently used conventional drugs (Barr *et al.*, 2007). Secondly the plants by secondary metabolic means contains a variety of herbal and non-herbal ingredients that are thought to act on a variety of targets by various models and mechanisms, given the multi-factorial pathogenicity of the disorders (Effiong *et al.*, 2015). According to Ebong *et al.* (2008) herbal therapies have pharmacological principles within themselves that work together in a dynamic way to produce therapeutic efficacy with minimum side effects.

Gongronema latifolium is an edible rain forest plant, native to the south eastern part of Nigeria. The common name is bushbuck. It has been reported to have much pharmacological importance. The *G. latifolium* is locally called "utazi" by Igbos, "arokeke" by Yorubas, and utasi" by the Efiks and Ibiobios in Nigeria (Ugochukwu and Babady, 2002). *Gongronema latifolium* belongs to the family of Asclepiadaceae. It is an edible rainforest plant native to the South East part of Nigeria and has widely used in folk medicine (Johnkennedy *et al.*, 2012). It is a herbaceous shrub with yellow

flowers and the stem that yields characteristic milky exudates when cut. Some phytochemicals such as β – sistosterol, lupenyl esters, pregnance ester, glycosides, essential oils and saponins have been isolated from this plant (Johnkennedy *et al.,* 2012). The Plant has been used as source of food, wealth and has helped to maintain a relatively disease-free state when properly utilized as herbal medicine (Analike and Joseph, 2015). Despite appreciable progress made in conventional anti-hyperlipidemic and oxidative stress management strategies, the search for plant-based products for the control of Hyperlipidemia continues and oxidative stress which are associated with a number of pathogens including dyslipidemia, atherosclerosis, diabetes mellitus, heart diseases, neurodegenerative diseases, cancer, among others which are risk factors for cardiovascular diseases (krauss *et al.,* 2000; Barr *et al.,* 2007).

This study is aimed at investigating the *in-vitro* antioxidant activities and *in-vivo* hypolipidemic effects of ethanol extracts of *G. latifolium* leaves in hyperlipidemic model of Wister albino rats.

Materials And Methods

Procurement of plant materials and animals

Fresh leaves of *Gongronema latifolium* used for this study was purchased from Ndoru market in Ikwuano Local Government Area of Abia State, Nigeria. The plant was identified by Mr. N. Ibe of the Forestry Department, College of Natural and Environment Management, Michael Okpara University of Agriculture, Umudike. The Wister albino rats were were procured from Department of Zoology, University of Nigeria, Nsukka, Enugu State. The animals were kept in well ventilated laboratory cages in animal house of Biochemistry Department, Michael Okpara University of Agriculture, Umudike.

Preparation of ethanol extract of *G. latifolium* leaves

The leaves were thoroughly washed in a clean tap water to remove dirt and debris and thereafter air dried at room temperature (27 °C ± 2 °C) for seven days to remove moisture until it was completely dry. The dried leaves were blended to a fine powder using a dry Moulinex super blender and stored in air-tight containers. Two hundred

grammes of the ground leaves was weighed using an electronic scale and afterwards soaked in 800 mL of 98% ethanol (*v*/*v*). To allow for proper mixing of the powder and the solvent, the mixture was agitated and then put in air-tight containers. The containers holding the mixtures were kept in the refrigerator at a temperature of 4 °C for 48 hr. Filtration of the mixture was accomplished first by using a cheesecloth, followed by the Whatman No. 1 filter paper (24 cm). The filtrate was concentrated using a rotary evaporator (model RE52A, Zhengzhou, China) to 10% of its original volume at a temperature of 37–40 °C. It was then concentrated to complete dryness in a water bath. The extract was afterwards refrigerated at 2–8 °C until when required for administration.

Collection of animals/ acclimatization

Fourteen (14) Wister albino rats (80-100g) of both sexes were obtained from the animal house of the Department of Zoology, University of Nigeria Nsukka. The animals were housed in a clean and dry aluminium cages at room temperature under standard conditions and were maintained at 12 hrs light and dark cycle. The animals were fed with standard pellet feed (vital feed, Jos Nigeria) and clean tap water during an acclimatization period of 14 days before experimentation.

Induction of hyperlipidemia

For the induction with high fat diet, the Wister albino rats were fed 15g each of the high fat diet for a period of 14 days. The composition of the high fat diet in g/1000g is as follows: maize (388.80), egg yolk (58.39), powdered groundnut cake (133.93), crayfish (21.58), vitamins (3.97), minerals (15.89), bone meal (19.87), cellulose (3.97), palm kernel oil (69.53), palm oil (69.53) and cornstarch (214.54). For comparison, a group of Wister albino rats (negative control) were maintained on normal diet (pelletized, vital feed). All the animals had free access to clean tap water. Body weight of the animals was measured after every 7 days. The experiment was conducted in accordance with the internationally accepted principles for laboratory animal use and care (NIH Guide, 1996).

Experimental design

The experiment consists of a total of fourteen Wister albino rats which were divided into seven (7) groups (A-G) of two (2) rats each.

Group A: the normal control. Group B: High fat fed rats maintained on high fat diet and normal saline (negative control). Group C: High fat fed rats treated with Simvastatin (a standard lipid lowering drug) at a dose of 10 mg/kg b.w (positive control). Group D: High fat fed rats treated with ethanol extracts of *G. latifolium* leaves at a dose of 500 mg/kg b.w. Group E: High fat fed rats treated with ethanol extracts of *G. latifolium* leaves at a dose of 250 mg/kg b.w. Group F: High fat fed rats treated with ethanol extracts of *G. latifolium* leaves at a dose of 125 mg/kg b.w. Group G: High fat fed rats treated with ethanol extracts of *G. latifolium* leaves at a dose of 62.25 mg/ kg b.w.

The ethanol extract of *G. latifolium* leaves and the standard drug were administered to the rats orally using gavage daily for 14 days. After 14 days of treatment, animals were sacrificed by cervical decapitation after a 12h fast.

Blood serum sample collection and preparation

Eighteen hours after the last feeding, the animals were sacrificed, sterilized dissecting scissors were used to open the thoracic cavity while 5.0 ml of blood was collected by cardiac puncture into a plain sample container using syringe. The serum gotten was subjected to further separation by centrifugation, using an MSE table top centrifuge (Buckinghamshire, England), set at 8000 rpm (revolutions per minute) for 15 min to ensure clear supernatant devoid of traces of red cells The serum samples collected were stored in a refrigerator at 4 °C for subsequent biochemical assays. All test analysis was run in triplicate and the average values were reported. The percentage of DPPH radical scavenging was calculated using the equation below (Krishnaiah *et al.*, 2011).

$$\% \text{ scavenging activity} = (A_{standard} - A_{sample}) / A_{standard} \times 100$$

Nitric Oxide Scavenging Activity

Nitric oxide (NO) is generated in biological tissues by specific nitric oxide synthases, which metabolises arginine to citrulline with the formation of NO via a five electron oxidative reaction. The

compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO. Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite), the quantities of which can be determined using griess reagent.

2ml of 10mM sodium nitroprusside dissolved in 0.5ml phosphate buffer saline (pH 7.4) is mixed with 0.5ml of samples at various concentrations (200, 100, 50, 25, 12.5 mg/ml). The mixture is incubated at 25°C, 0.5ml of the incubated solution is withdrawn and mixed with 0.5 ml of griess reagent. (1.0ml sulfanilic acid reagent (0.33% of 20% glacial acetic acid) at room temperature for 5minutes with 1ml of naphylethylenediamine dichloride(0.1%w/v). The mixture is then incubated for 30minutes at room temperature and its absorbance measured at 546nm.

The amount of nitric oxide radical inhibition is calculated thus:

% Inhibition activity= $(A_{standard} - A_{sample}) / A_{standard} \times 100$

Determination of Anti Lipid Peroxidation

The extent of lipid peroxidation using rat brain homogenate measured in terms of formation of thiobarbituric acid reactive substances. Different concentrations (200, 100, 50, 25, 12.5 mg/ml) of the plant extract were made up with ethanol expressed in terms of dry weight. These samples were individually added to the brain homogenate (0.5ml). This mixture was incubated with 0.15M KCL (100ul).

Lipid peroxidation was initiated by adding 100ul of 15 Mm $FeSO_4$ solutions. The reaction mixture was incubated at 37°C for 30 minutes. An equal volume of TBA: TCA (1:1, 1ml) was added to the above solution followed by the addition of 1ml vitamin C. this final volume was heated on a water bath for 20 minutes at 80°C and cooled, centrifuged and absorbance read at 532nm.

The % inhibition of lipid peroxidation was calculated thus;

% Inhibition activity = $(A_{standard} - A_{sample}) / A_{standard} \times 100$.

Ic_{50} Calculation

IC_{50} values which represents the concentration of *G. latifolium* leaves that caused 50% inhibition were calculated from a scattered plot of % inhibition against concentration (mg/ml), using the equation $y = mx + c$: where y = 50% inhibition, x = IC_{50} value, m = slope and c = intercept, all from the plot using MS Excel or graph sheet.

Statistical Analysis

Values for lipid profile, weight gain/loss and antioxidant activities were expresses as mean ± standard deviation. The data obtained were statistically analysed using one way analysis of variance (ANOVA) with Duncan's multiple comparison post HOC tests to compare the level of significance between control and experimental groups. The values $p<0.05$ were considered significant.

Results

Plant extract yield

The maceration of the powdered plant sample in ethanol gave a total yield of 14%

Antioxidant results:

Free radical scavenging activity of extracts of *G. latifolium* was investigated. They are represented as triplicate mean ± SD as shown in Tables 1 to 3 below. Table 1revealed the result of DPPH percentage scavenging activity of ethanol extract of *G. latifolium* leaves. From Table 1, there is a significant ($p< 0.05$) decrease in percentage inhibition of DPPH when compared against the standard group.

Table 2 showed the result of anti-lipid peroxidation activity of ethanol extract of *G. latifolium* leaves. From the table, there is a significant ($p<0.05$) decrease in anti-lipid peroxidation when compared against the standard group

The result of nitric oxide inhibition activity of ethanol extract of *G. latifolium* at different concentrations is as shown in Table 3. From the Table, there is a significant ($p< 0.05$) decrease in percentage inhibition of nitric oxide with concentration when compared against the standard group.

Lipid Profile

The Lipid profile of ethanol extract of *G. latifolium* was investigated. They are represented as triplicate mean $\pm$ SD as shown in Tables 4 to 7 below.

Table 4 showed the effect of ethanol extract of *G. latifolium* leaves on total cholesterol. From the table, there is a significant decrease ($p<0.05$) in the T-cholesterol levels when the treated groups (positive control, 500mg/kg, 250mg/kg, 125mg/kg, 62.5mg/kg) are compared to the untreated groups (Normal ctrl, Negative ctrl) and a significant increase ($p<0.05$) in T-cholesterol levels of the groups given the extract when compared to the Simvastatin group (positive control), T-cholesterol of the extract treated groups also decreases significantly ($p<0.05$) with an increase in concentration of the extract .

Table 5 revealed the effect of ethanol extract of *G. latifolium* leaves on triglycerides level.

From Table 5, there is a significant decrease ($p<0.05$) in the TAG levels when the treated groups (positive control, 500mg/kg, 250mg/kg, 125mg/kg, 62.5mg/kg) are compared to the untreated groups (Normal ctrl, Negative ctrl) and a significant increase ($p<0.05$) in TAG levels of the groups given the extract when compared to the Simvastatin group (positive control), the TAG of the extract treated groups also decreases significantly ($p<0.05$) with an increase in concentration of the extract.

Table 6 showed the effect of ethanol extract of *G. latifolium* leaves and Simvastatin on High density lipoprotein- cholesterol levels. From the table, there is a significant increase ($p<0.05$) in the HDL-c levels when the treated groups (positive control, 500mg/kg, 250mg/kg, 125mg/kg, 62.5mg/kg) are compared to the untreated groups (Normal ctrl, Negative ctrl) and a significant decrease ($p<0.05$) in HDL-c levels of the groups given the extract when compared to the Simvastatin group (positive control), the HDL-c of the extract treated groups also increases significantly ($p<0.05$) with an increase in concentration of the extract.

The effect of ethanol extract of *G. latifolium* leaves on Low Density Lipoprotein- cholesterol levels is as revealed in Table 7. From Table

7, there is a significant decrease ($p<0.05$) in the LDL-c levels when the treated groups (positive control, 500mg/kg, 250mg/kg, 125mg/kg, 62.5mg/kg) are compared to the untreated groups (Normal ctrl, Neg. ctrl) and a significant increase ($p<0.05$) in LDL-c levels of the groups given the extract when compared to the Simvastatin group (Positive control), the LDL-c of the extract treated groups also decreases significantly ($p<0.05$) with an increase in concentration of the extract.

Discussion

Hyperlipidemia is a serious condition of elevated lipid levels in the body that ultimately lead to the development and progression of various cardiovascular diseases (CVDs). From the result, the total cholesterol, triglyceride and low density lipoprotein cholesterol (LDL-c) was significantly higher ($p<0.05$) and HDL-c was significantly lower ($p<0.05$) in the negative control compared to the treated groups. This shows that the induction of hyperlipidemia by feeding a high fat diet to the experimental animals caused significant metabolic changes in them.

On comparing between the positive control (Simvastatin treated) and the *G. latifolium* extract treated groups shows that they both acted on the lipid profile of the animals but there was a significant increase ($p<0.05$) in the TC, TAG, LDL-c levels and a significant decrease in the HDL-c levels when the extract treated group is compared to the positive control. These effects were similar to those effects showed by Simvastatin (standard lipid lowering drug). At the highest concentration of the extract (500mg/kg), there was only a slight difference in the range of values of the LDL-c levels (28.7 ± 0.3) when compared to the positive control (26.8 ± 0.7). This could be as a result of the similar mechanism of action of the extract and the standard drug.

When compared between the extract treated groups, there was a significant increase ($p<0.05$) among the groups with an increase in the effect of the extract relative to the increase in concentration of the extract. The HDL-c levels was increased to 33.6 ± 0.5 for 62.5mg/kg and 43.7 ± 1.5 at 500mg/kg, also, LDL-c levels was reduced to 51.7

±1.1 at 62.5 mg/kg and 28.7 ± 0.3 at 500mg/kg . This implies that an increase in concentration of the ethanol extract of *G. latifolium* leaves increases its hypolipidemic effect (500mg/kg > 250mg/kg > 125mg/kg > 62.5mg/kg) (Khanna *et al.*, 2002).

Dietary cholesterol has been shown to reduce fatty acid oxidation, which in turn increases the levels of hepatic and plasma triglycerides (Davis *et al.*, 1982). Possible mechanism of hypocholesterolemic effect could be by conversion to bile acid since decrease in HDL may affect the reverse transport of cholesterol to the liver for biosynthesis. The role of lipids particularly cholesterol in the aetiology of coronary heart diseases (CHD) is well documented in literature (Davies, 2000). In fact, part of treatment and management of CHD is to reduce cholesterol and replace saturated with unsaturated or polyunsaturated fats in the diet (Villaseñor, 2016). Even drugs like vastatin series act by inhibiting cholesterol synthesis and thus increasing the degradation (Viktorinova *et al.*, 2016). Both LDL and HDL play important roles in cholesterol metabolism. For example, LDL content in the arterial intima accounts for cholesterol build up due to certain reasons like the small size, fragile nature, and easy of oxidation.

Increased permeability of the endothelium and increased intimal retention of LDL appear to play an important role in atherosclerosis. LDL particles interact with particular constituents of the intima, particularly the extracellular matrix. Chondroitin sulphate proteoglycans produced by smooth muscle cells in the arterial wall react with apolipoprotein B on the surface of lipoprotein particles, thereby increasing the retention of LDL (Sodipo *et al.*, 2011; Zhen *et al.*, 2016). This implies that LDL easily breaks down depositing cholesterol within the arterial wall which later develops into atherosclerotic plague (Davies 2000). However, HDL has higher molecular weight, more stable with antioxidant properties, and transport cholesterol from the arterial intima back to the liver (Reverse transport) where it is used for biosynthesis. Thus, higher levels of HDL favour cholesterol clearance (Nwachukwu and Iweala, 2013).

Therefore high levels of LDL-c (60.8± 1.1) greater than that of HDL-c (31.2 ± 1.2) as seen in the negative control is a crucial risk factor for cardiovascular diseases like atherosclerosis and this may be seen with

an increased cholesterol level that may be accompanied by an increased triglyceride levels (Tables 6 and 7). From the results, it may be said that the hypolipidemic activity of the extract after daily oral dose of administration of the extract may be due to a number of mechanisms. This may include; Inhibition of HMG-COA reductase enzyme activity which is responsible for synthesis of cholesterol by the liver, a similar way of Simvastatin action; Stimulation of cholesterol – 7-alpha- hydroxylase, which converts cholesterol into bile acids; Inhibition of cholesterol absorption from the intestine due to the formation of complexes with compounds such as saponins, glycosides and flavonoids (Gundamaraju *et al.*, 2014).

A reduction in triglyceride levels may be due to decreased lipogenesis, increased lipolytic activity by inhibition of hormone sensitive lipase or lipogenic enzymes or activation of lipoprotein lipase, as has been proposed for Simvastatin and some plant exhibiting hypolipidemic activity. Recent studies also show that HDL-c help promotes the reverse cholesterol transport pathway, by inducing an efflux of excess accumulated cellular cholesterol and prevents the generation of an oxidatively modified LDL, counteracting LDL- oxidation (Daradka *et al.*, 2017)

G. latifolium has been reported to contain saponins and other phytochemicals (Morebise *et al.*, 2002; Francis *et al.*, 2013). Saponin has the ability to reduce lipid uptake at the gut by binding with cholesterol and resulting to poor absorption and reduced endohepatic circulation of bile acids. Increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequently lowering of the plasma cholesterol and this may be attributed to the hypocholesterolemic effect of the extract. This is in consonance with the work of these scientists (Ugochukwu *et al.*, 2003; Analike and Joseph, 2015; Sylvester *et al.*, 2015) who reported that the phytochemicals present in *G.latifolium* reduce LDL-c involved in depositing fat in the arteries, prevent blood clotting which can reduce the risk of heart attack or stroke.

Thus, the results suggest that the oral administration of ethanol extract of *G. latifolium* leaves at different doses studied; significantly ($p < 0.05$) reduced the plasma triglyceride biosynthesis and favours the redistribution of cholesterol among lipoprotein molecules.

Ethanol extract of *G. latifolium* leaves may probably play an anti-atherogenic role through the inhibition of lipids oxidation as well as the elevation of HDL-cholesterol.

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and aging. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation and by many other mechanisms and thus prevent diseases (Davies, 2000; Ramadan and Schaalan, 2011). From the results of the evaluation of the *in-vitro* antioxidant activity of *G. latifolium* leaves, the percentage (%) inhibition/ scavenging activities by DPPH, anti-lipid peroxidation and nitric oxide showed a linear increase with increasing concentrations of the extracts. DPPH scavenging activity increased significantly ($p<0.05$) with an increase in concentration of the extract from 31.82 ± 5.9 for 12,5 mg/ml to 85.21 ± 3.3 for 200mg/ml when compared with the standard whose percentage activity was 99.01 ± 0.5

When compared to the standard, at 100mg/ml of both *G. latifolium* extract and the standard, percentage DPPH scavenging activity showed 73.52 ± 2.5 for *G. latifolium* leaves and 99.05 for the standard. Anti-lipid peroxidation activity shows 53.99 ± 5.0 for *G. latifolium* leaves at 100mg/ ml and 99.41 ± 0.1 at 100mg/ml of the standard. Also, *G. latifolium* leaves showed percentage nitric oxide inhibition activity of 59.13 ± 4.6 when compared with the standard (96.68 ± 0.4) and this inhibition activity increases with an increase in the concentration of the extract. On a comparative basis, the extract showed better activity in quenching of DPPH scavenging activity compared to other activities.

DPPH is a relatively stable radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. From the result, it may be postulated that *G. latifolium* leaves reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles. DPPH radicals react with suitable reducing agents, the electron pairs off and the solution loses colour depending on the number of electron taken up (Ala *et al.*, 2006).

The peroxidation of membrane lipids initiated by oxygen radicals may lead to cell injury. Initiation of lipid peroxidation by ferrous sulphate takes place through OH radical by Fenton reaction thereby initiating a number of oxidative reactions. The results obtained in the present study may be attributed to several reasons through, scavenging of OH or superoxide radicals by changing the ratio of Fe^{3+}/Fe^{2+}; reducing the rate of conversions of ferrous to ferric or by chelation of iron itself. The antioxidant activity of the extract may probably be due to the rapid and extensive degradation of the antioxidant principles.

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases. In the present study, the nitrite produced was reduced by the ethanol extract of *G. latifolium* leaves. This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. This finding is in conformity with the reports of other researchers (Zin *et al.*, 2002; 2006; Ala *et al.*, 2006).

Reactive oxygen species (ROS) have been considered deleterious to cell function and there is good evidence to suggest that they play a role in the pathophysiology of cardiac diseases state (Dhalla and Temsah, 2000). One of the strategies used to assess the role of oxidative stress in the pathogenesis of cardiac dysfunction has been to expose isolated cardiac tissues to a defined oxidation stress condition and study the resulting effects. Most cardiovascular diseases are secondary to atherosclerosis, a disease of the arteries involving a local thickening of the vessel wall. Although a high level of plasma cholesterol is considered to trigger atherosclerosis, the oxidation of cholesterol seems to be a necessary step (Diao *et al.*, 2016). In fact, uptake of oxidized low density lipoprotein was shown to be an early event leading to the development of atherosclerosis. Oxidized LDL and oxidized lipoproteins have been reported to stimulate 0_2^- formation leading to apoptosis of cells in the umbilical vascular wall; this was prevented by treatment with antioxidants.

In cultured human coronary artery smooth muscle cells, low levels of

the involvement of oxidative stress in the pathogenesis of atherosclerosis. High levels of oxidized LDL were apoptotic implicating the additive role of ROS in increased plaque vulnerability; this effect was reduced by antioxidants. Patients with atherosclerosis and hypercholesterolemia showed higher susceptibility of LDL to oxidative in comparison to patients treated with lipid –lowering agents (William and Boden, 2007)

Many phenolic compounds have been shown to have antioxidant activities *in vitro* and several observational studies support their role in potentially protecting against cardiovascular diseases (Trease and Evans, 1989; Iwara *et al.*, 2015). From the present result, *G. latifolium* leaves which possess antioxidant effect may serve protective effect against cardiovascular diseases; by reducing LDL oxidation and reducing cholesterol levels. This is in agreement with the findings of Ugochukwu *et al.* (2013) who reported the antioxidant properties of *G. latifolium*.

Conclusion

From the results obtained in this study, it has been ascertained that the ethanolic leaf extract of *G. latifolium* possesses antioxidant properties which could be attributed to bioactive compounds present in the plant hence a good candidate for use as phytotherapeutics to reduce high cholesterol levels and other cardiovascular diseases. However, further studies on the molecular mechanism underlying its effect on functional lipids need to be investigated.

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Table 1: Shows the result of DPPH percentage scavenging activity of ethanol extract of *G. latifolium* leaves

Concentration (mg/ml)	% inhibition activity	
	% inhibition	Standard (IC_{50})
200	85.21± 3.3	
100	73.52± 2.5	99.01± 0.5
50	52.72 ± 7.4	
25	37.06± 6.5	
12.5	31.82± 5.9	

Table 2: Shows the result of anti-lipid peroxidation activity of ethanol extract of *G. latifolium* leaves.

Concentration (mg/ml)	% inhibition activity	
	% inhibition	Standard (IC_{50})
200	82.44± 2.6	
100	53.99± 5.0	99.41± 0.1
50	38.81 ± 2.7	
25	28.04± 5.6	
12.5	14.44± 15.0	

Table 3: The result of nitric oxide inhibition activity of ethanol extract of *G. latifolium* at different concentrations:

Concentration (mg/ml)	% inhibition activity % inhibition	Standard (IC_{50})
200	72.23± 3.3	
100	59.13± 4.6	98.68± 0.4
50	41.57 ± 1.5	
25	26.19± 2.1	
12.5	19.90± 7.1	

Table 4: Shows the effect of Ethanol Extract of *G. latifolium* leaves on total Cholesterol.

Groups	**Total cholesterol(mg/dl)**
Normal control	111.5±1.8
Negative control	97.5± 0.8
Positive control	53.5± 3.8
500mg/kg	61.7± 1.1
250mg/kg	77.1± 1.5
125mg/kg	88.9± 0.1
62.5mg/kg	92.8± 1.1

Table 5: Shows the effect of ethanol extract of *G. latifolium* leaves on triglycerides level.

Groups	**Triglyceride (mg/dl)**
Normal control	90.3±2.4
Negative control	79.5±1.8
Positive control	37.2±1.9
500mg/kg	43.2±0.6
250mg/kg	54.8±0.2
125mg/kg	64.2± 0.5
62.5mg/kg	69.1±1.2

Table 6: Shows the effect of ethanol extract of *G. latifolium* leaves and Simvastatin on High density lipoprotein- cholesterol levels.

Groups	HDL-c (mg/dl)
Normal control	30.3±2.7
Negative control	31.2±1.2
Positive control	47.2±1.6
500mg/kg	43.1±1.5
250mg/kg	39.2±1.2
125mg/kg	36.5± 0.4
62.5mg/kg	33.6±0.5

Table 7: Shows the effect of ethanol extract of *G. latifolium* leaves on Low Density Lipoprotein- cholesterol levels.

Groups	LDL-c (mg/dl)
Normal control	71.5±1.0
Negative control	60.8±1.1
Positive control	26.8±0.7
500mg/kg	28.7±0.3
250mg/kg	31.7±1.2
125mg/kg	41.5± 0.9
62.5mg/kg	51.7±1.1