Original Research Article

Cognitive Antihyperlipidemic and Antioxidant Activities of Bitter leaf (*Vernonia amygdalina*) and Scent leaf (*Ocimum gratissimum*)

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A b s t r a c t

Oxidative stress manifests an imbalance in the production and the detoxification of free radicals. Free radicals cause oxidative degradation of biomolecules like lipids, proteins, nucleic acids, and carbohydrate molecules, thereby compromising cell integrity and function and leading to health deterioration from a range of metabolic anomalies that cause diseases and ailments including cardiovascular diseases (CVD), hypertension, stroke and cancer. Both bitter leaf (VA) and Scent leaf (OG) have been medicinally useful in the cure of diseases in different cultures.The addition of scent leaf as additive in preparation of delicacies in Nigeria has prompted this study to determine the cognitive activities of both plants to repair the damages caused by high fat diet present, by evaluating the antidyslipidemic activitiesas well as their antioxidant potentials. The results obtained revealed that both plants have antioxidant abilities when subjected to standard antioxidant evaluations although VA was significantly higher (p<0.05) when compared with OG, however the cognitive effects showed that the antioxidant potentials of VA was depressed on the addition of OG. The results obtained from the modulation of lipid in albino rats challenged with hyperlipidemia showed that both VA and OG have a positive effect to lower LDL-C, TAG, TCHOL and increase HDL-C thus potentiating their antidyslipidemic ability and protective role against dyslipidemic disorders. Similarly, the cognitive effect showed reduction in the activities of VA while the activity of OG was boosted. The high phospholipid contents of the selected tissues upon treatments with the leaf extracts also showed that both VA and OG can have ameliorating effects on coronary heart disease, inflammation or cancer by enhancing membrane integrity. It is noteworthy that both VA and OG have good potential in the ability to reverse induced dyslipidemia and in spontaneous cellular lipid, while the addition causes a reduction in the activities of VA.

Keywords: Bitter leaf (VA); Scent leaf (OG); LDL-C; TAG; TCHOL; HDL-C; Dyslipidemia; Antioxidant; High fat diet

Introduction

Cardiovascular disease is one of the leading causes of death. One of the major risk factors for the development of cardiovascular disease is dyslipidemia, which may be primarily associated with hypertension, diabetes mellitus and obesity [1]. Dyslipidemia usually involves elevated plasma levels of triglycerides, total, LDL and VLDL cholesterol and a low level of HDL cholesterol [1]. Therefore, any nutritional and pharmacologic intervention that improves or normalizes abnormal lipid metabolism may be useful for reducing the risk of cardiovascular diseases [2] and dyslipidemic disorders. Several drugs are at present, available for the management of dyslipidemia. However, there is renewed interest in the use of herbal products [3]. This may be attributable to the down turn in the economy, as traditional medicine is perceived to be a cheaper means of treatment [4]. WHO in 1991 developed guidelines for the assessment of herbal medicine [5].

Among the plants whose parts have found wide pharmacological applications are bitter leaf (*Vernonia amygdalina)* and scent leaf (*Ocimum gratissimum)*.Bitter leaf (VA), a member of the Asteraceae family, is a small shrub that grows in the tropical Africa. Bitter leaftypically grows to a height of 2-5 m. It is commonly called bitter leaf in English because of its bitter taste. The cooked leaves are a staple vegetable in soups and stews of various cultures throughout equatorial Africa. African common

names include Grawa (Amharic), Ewuro (Yoruba), Etidot (Ibibio), Onugbu (Igbo), Ityuna (Tiv), Oriwo (Edo), Chusar-doki (Hausa), Mululuza (Luganda), Labwori (Acholi), Olusia (Luo), and Ndoleh (Cameroon). Scent leaf (OG), also known as Clove Basil, African Basil and Wild Basil, is a species of *Ocimum*. It is native to Africa, Madagascar, southern Asia, and the Bismarck Archipelago, and naturalized in Polynesia, Hawaii, Mexico, Panama, West Indies, Brazil, and Bolivia. It belongs to the family Lamiaceae. It is commonly called 'alfavaca' and is cultivated in many gardens around village huts in Nigeria for its medicinal and culinary uses.

The parts of the plants mostly used are the leaves, usually fresh to obtain optimum result [6]. VA is used for a wide range of ailments such as constipation, fever, purgative, worm remover and against urinary inflammation in local medicine. OG has been used as a medicinal plant in the treatment of headache, diarrhea, and wart and kidney function [7]. VA and OG are used in the treatment of diabetes by tradomedical practitioners [8], the herbs are also used as insect repellant, smell disguiser and for colic [9], antimicrobial [10], antifungal [11], antiprotozoal and antimalarial [12]. Also *V. amygdalina* leaf possesses both hypoglycemic and hypolipidemic effect and is capable of normalizing other biochemical and hematological abnormalities associated with diabetes mellitus and thus could be prescribed as adjunct to dietary and main therapy for diabetes mellitus [13]. Therefore, this research work was aimed at comparing the lipid modulating and antioxidant potentials of *Vernonia amygdalina* and *Ocimum gratissimum*leaves on the antioxidant and lipidomic status of albino rats challenged with hyperlipidemia.

Materials and Methods

Materials

Reagents and Chemicals

Reagents and Chemicals used in this experiment were obtained from different sources such as British Drug House (BDH) and Sigma limited and were all of good analytical grades. All the solutions, buffers and reagents were prepared using glass distilled water.

Sample Preparation

Fresh *Vernonia amygdalina* (bitter leaf) and *Ocimum gratissinum* (scent leaf) leavesof 500g each were separately washed gently, without squeezing, to remove dirt and sundried. The air-dried leaves were separately blended to a coarse powder in a mortal and extracted with distilled water as follows: Coarse powder (100g) were separately soaked in 250ml distilled water in a beaker and the mixture made to vortex (using a vortex mixer) for 24hours before filtration.The filtrate were evaporated in a vacuum oven at

55^0C to obtain a solid residue (*Vernonia amygdalina* leaf, *Ocimum gratissinum* leaf).These were then reconstituted in distilled water to prepare a concentration of 5g/ml for each sample. Extracts were kept at 4^0C.

In Vitro Antioxidant Assays

Since the evaluations of antioxidant activities were quantitative analyses, all assays were performed in triplicate. For each test performed, 500μg/ml concentrations of the extracts and standards were prepared.

Assay for Total Antioxidant Activity

To the reagent solution; sulphuric acid (0.6 M), sodium phosphate (28 mM) and ammonium molybdate (4 mM); 0.3 ml of sample was added and incubated at 95^0 C in a water bath for 90 min. After cooling to room temperature absorbance was recorded at 765 nm against reagent blank. The absorbance of the sample was extrapolated on the ascorbic acid standard curve to obtain concentration of the sample in mg/ml then the total antioxidant activity (mg/g ascorbic acid equivalence) was calculated thus:

Y= MX + C

Y= Absorbance of the standard

M= Slope of the graph

X= Calculated concentration, which is the total antioxidant activity (mg/g).

C= Intercept

Total antioxidant activity (TAA) mg/g = X = (Y-C)/M

Reducing Power Assay

The reducing power of the extracts was determined according to the method of [14].Extract (0.5 ml) was mixed with 1.25 ml each of phosphate buffer and potassium ferricyanide ($C_6N_6FeK_3$). The mixture was incubated at 50^0 C for 20 min. Trichloroacetic acid (1.25 ml) was then added and the mixture centrifuged at 3000 rpm for 10 min. Thereafter, 1.25 ml of the upper layer of the solution was mixed with 1.25 ml of distilled water and 0.25 ml of $FeCl_3$. The absorbance was read at 700 nm. Higher absorbance of the reaction mixture indicates greater reductive potential.

DPPH Radical Scavenging (1, 1-diphenyl-2-picrylhydrazyl) Activity Assay

The DPPH radical scavenging activity of the extracts was evaluated according to the method described by Leong and [15]

% scavenging activity = ((Ac − As)/Ac) x 100. Where Ac is the absorbance of control and As the absorbance of the extract.

Metal Chelating Activity Assay

The metal chelating activity was determined according to the method of [16].

The Fe^{2+} chelating capacity was calculated thus: Fe^{2+} chelating activity (%) = ((Ac − As)/ Ac) x 100

Hydroxyl Radical Scavenging Activity Assay

The hydroxyl radical scavenging activity was determined according to the method of [17].

OH scavenging activity (%) = ((Ac − As)/ Ac) x 100

Assay of 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulphonic Acid) (ABTS) Radical Scavenging Activity

ABTS radical scavenging activity of the plant extract was determined according to the method of [18]

% scavenging activity = ((Ac − As)/Ac) x 100

Where Ac is the absorbance of control and As the absorbance of the extract.

Experimental Design

A total of 80 male wistar albino rats (200±20g body weight) were used and treated as directed by 'The Guide for the Care and Use of Laboratory Animals'. The animals were maintained at 25°C in a well-ventilated animal house on a 12:12 h light-dark cycle with free access to standard rodent laboratory chow and water *ad libitum*. Prior to use, animals were acclimatized for 14days.Hyperlipidemia was induced and experiment design according to [19] by administering high fat diet for four weeks, after which they were segregated into groups followed by oral administrations of *V. amygdalina* or *O. gratissimum* or combined *V. amygdalina* and *O. gratissimum* aqueous leafextract for two weeks. 200mg/kg BWT of leaves were administered twice daily for both leaf extracts while 100mg/kg BWT from each of the extracts were administered for combined effect. Each group contained five (5) rats.Animals were sacrificed by cervical dislocation, blood samples collected via cardiac puncture into heparinized tubes as well as separate tubes for plasma separation. Brain, heart, liver and kidney were quickly collected, blotted free of blood and tissue fluid and collected under cold conditions using pre-cooled Petri-dishes, rinsed twice in ice cold normal saline (1.15% KCl) solution, weighedand were homogenized in ice cold 5 % w/v sodium phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenates were stored at 4 °C and then used for biochemical analysis. CNT 7DT-Control group after 7-day time; CNT 14DT-Control group after 14-day time; HYPL 7DT-Hyperlipidemic group without treatment for 7-day; HYPL+B.L 7DT- Hyperlipidemic group with *V. amygdalina* treatment for 7-day; HYPL+B.L 14DT- Hyperlipidemic group with *V. amygdalina* treatment for 14-day; HYPL+S.L 7DT-Hyperlipidemic group with *O. gratissimum* treatment for 7-day; HYPL+S.L 14DT- Hyperlipidemic group with *O. gratissimum* treatment for 14 days; HYPL+B.L+S.L. 7DT- Hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 7-day; HYPL+B.L+S.L. 14DT- Hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 14-day; B.L 7DT- Non-hyperlipidemic group with *V. amygdalina* treatment for 7-day; B.L 14DT- Non-hyperlipidemic group with *V. amygdalina* treatment for 14-day; S.L 7DT- Non-hyperlipidemic group with *O. gratissimum* treatment for 7-day; S.L 14DT- Non-hyperlipidemic group with *O. gratissimum* treatment for 14-day; B.L+S.L 7DT- Non-hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 7-day and B.L+S.L 14DT-Non-hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 14-day

Biochemical Evaluations

Assay of Catalase Activity:The activity of CAT was measured using its perioxidatic function according to the method of [20]. The absorbance was read at 550 nm in a spectrophotometer. Results are expressed as micromoles of formaldehyde produced/mg protein.

Assay of Superoxide Dismutase Activity: SOD activity was assayed by the method of [21]. Amount of chromogen formed was measured by recording color intensity at 560 nm. Results are expressed as units/mg protein.

Evaluation of Lipid Peroxidation: The extent of lipid peroxidation was estimated in terms of thiobarbituric acid reactive substances (TBARS), using malondialdehyde (MDA) as standard by method of [22]. The absorbance was read at 535 nm and the MDA concentration of the sample calculated using extinction coefficient of 1.56×10^5 M^{-1} Cm^{-1} and expressed as μmol/g Hb erythrocyte and μmol/g tissue.

Extraction of Lipids from Animal Tissues: 0.2g of organs was homogenized in about 80% of 1.8ml of chloroform-methanol mixture and homogenized. The homogenate was centrifuged at 4000rpm per minutes for 10mins and supernatant was recovered.0.1ml of 0.05M of KCl was added to remove other nonpolar components of the supernatant from the mixture and vortexed at room temperature for 5 minutes.The mixture was centrifuged again and the supernatant was taken and analyzed for lipid.

Evaluation of Triglyceride: Triacylglycerol was determined by enzymatic colorimetric method described by

Evaluation of Total Cholesterol: Cholesterol Determination using the manufacturer protocol of

Randox laboratory limited, United Kingdom.

Evaluation of Phospholipid: 20μl of chloroform methanol extract of spleen was diluted 5 folds and evaporated to dryness at 60^{0}C. After cooling, 2ml of chloroform was added to the extract and vortexed.2ml of ammonium ferothiocynate was also added. The mixture was vortexed for 1mins and left for 10mins for phases to separate. Chloroform layer (lower layer) was removed and absorbent was read at 488nm.Blank: 2ml of chloroform and 2ml of ammonium ferrothiocyanate was added together and vortexed for 1mins. It was then left for 10mins for phase to separate chloroform layer was removed and zeroed at 488nm

Evaluation of HDL-cholesterol concentration: The precipitation was carried out using the manufacturer protocol of Randox laboratory limited, United Kingdom.

LDL Cholesterol (mg/dl) = Total Cholesterol – Triglycerides/5 – HDL Cholesterol

Statistical Analysis

The results were pooled and expressed as mean ± standard deviation. One way analysis of variance (ANOVA) was used to analyze the results and Duncan multiple test was used for the post hoc (DMRT). Statistical package for Social Science (SPSS) 17.0 for Windows was used for the analysis. The significance level was set at $p < 0.05$.

Results

Table 1 showed the in vitro antioxidant activities of *V. amygdalina* (BL) and *O. gratissimum* (SL) as well as their combined effect. The assays of antioxidant activities were typified by total antioxidant activity, ABTS and DPPH radicals scavenging activities, metal chelating activity, reducing power and hydroxyl radical scavenging activities.

Table 1 showed the in vitro antioxidant activities of *V. amygdalina* (BL) and *O. gratissimum* (SL).

500µg/ML	TOTAL ANTIOXIDANT (MG/GAAE)	ABTS RADICAL SCAVENGING(% ACTIVITY)	DPPH RADICAL SCAVENGING(% ACTIVITY)	METAL CHELATING(% ACTIVITY)	REDUCING POWER (ABSORBANCE)	HYDROXYL RADICAL SCAVENGING(%ACTIVITY)
V. amygdalina + *O. gratissimum*	85.25±2.81[b]	78.92±4.92[b]	77.11±8.34[b]	62.81±3.22[b]	1.723±0.09[ab]	73.91±3.99[b]
V. amygdalina	101.42±3.82[c]	85.62±4.11[c]	82.18±6.82[c]	75.92±1.93[c]	2.16±0.32[bc]	85.66±5.83[c]
O.gratissimum	76.57±6.44[a]	69.13±2.15[a]	64.36±7.38[a]	57.36±1.98[a]	1.23±0.034[a]	65.34±2.83[a]
ASCORBICACID	-	-	94.72±6.35[d]	-	2.637±0.05[c]	-
MANNITOL	-	-	-	-	-	96.34±9.16[d]
EDTA	-	-	-	93.17±3.92[d]	-	-
TROLOX	-	90.61±2.81[d]	-	-	-	-

Values are expressed as mean (n = 3). Values are expressed as mean±standard deviation. Values with different superscript are significantly different (p<0.05).
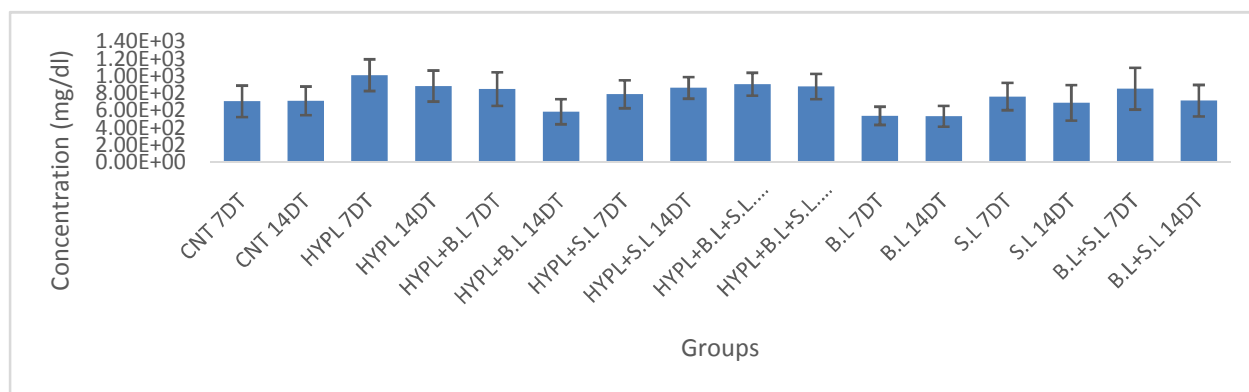

Figure 1: Effects of extracts on serum lipid peroxidation MDA concentration (mg/dl). Values are expressed as mean (n = 5).

Each value represents mean (n=5). CNT 7DT-Control group after 7-day time; CNT 14DT-Control group after 14-day time; HYPL 7DT-Hyperlipidemic group without treatment for 7-day; HYPL+B.L 7DT- Hyperlipidemic group with *V. amygdalina* treatment for 7-day; HYPL+B.L 14DT- Hyperlipidemic group with *V. amygdalina*

treatment for 14-day; HYPL+S.L 7DT- Hyperlipidemic group with *O. gratissimum* treatment for 7-day; HYPL+S.L 14DT- Hyperlipidemic group with *O. gratissimum* treatment for 14 days; HYPL+B.L+S.L. 7DT- Hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 7-day; HYPL+B.L+S.L. 14DT- Hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 14-day; B.L 7DT- Non-hyperlipidemic group with *V. amygdalina* treatment for 7-day; B.L 14DT- Non-hyperlipidemic

group with *V. amygdalina* treatment for 14-day; S.L 7DT- Non-hyperlipidemic group with *O. gratissimum* treatment for 7-day; S.L 14DT- Non-hyperlipidemic group with *O. gratissimum* treatment for 14-day; B.L+S.L 7DT- Non-hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 7-day and B.L+S.L 14DT-Non-hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 14-day
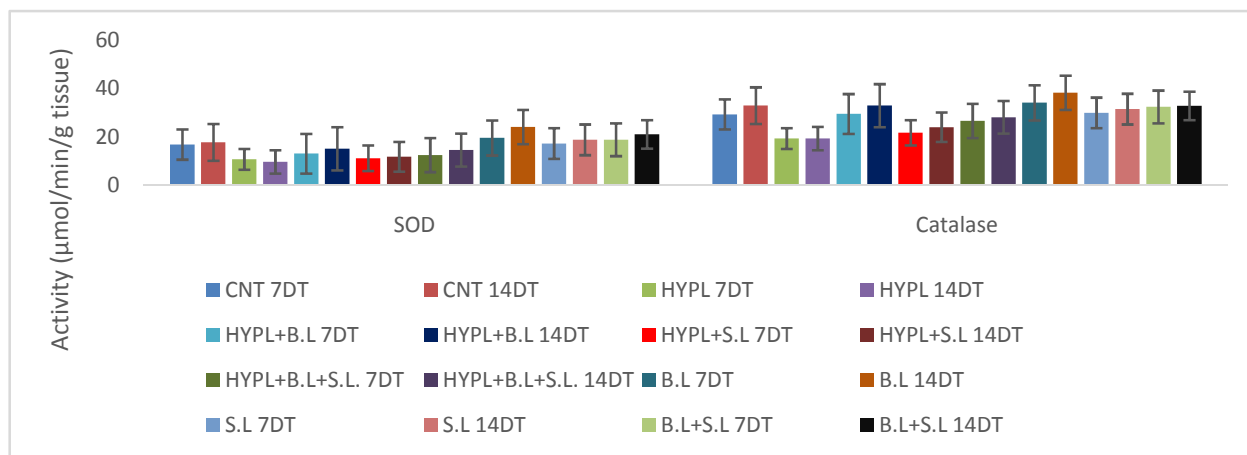

Figure 2: Alterations of Activities of Superoxide dismutase and Catalase in Serum by hyperlipidemia and antihyperlipidemic activities of extracts of the leaves of *V. amygdalina* and *O. gratissimum*. Each value represents mean (n=5).

CNT 7DT-Control group after 7-day time; CNT 14DT-Control group after 14-day time; HYPL 7DT-Hyperlipidemic group without treatment for 7-day; HYPL+B.L 7DT- Hyperlipidemic group with *V. amygdalina* treatment for 7-day; HYPL+B.L 14DT- Hyperlipidemic group with *V. amygdalina* treatment for 14-day; HYPL+S.L 7DT- Hyperlipidemic group with *O. gratissimum* treatment for 7-day; HYPL+S.L 14DT- Hyperlipidemic group with *O. gratissimum* treatment for 14 days; HYPL+B.L+S.L. 7DT- Hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 7-day; HYPL+B.L+S.L. 14DT- Hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 14-day; B.L 7DT-Non-hyperlipidemic group with *V. amygdalina* treatment for 7-day; B.L 14DT- Non-hyperlipidemic group with *V. amygdalina* treatment for 14-day; S.L 7DT- Non-hyperlipidemic group with *O. gratissimum* treatment for 7-day; S.L 14DT- Non-hyperlipidemic group with *O. gratissimum* treatment for 14-day; B.L+S.L 7DT- Non-hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 7-day and B.L+S.L 14DT-Non-hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 14-day

The activity of catalase (Figure. 2) with respect to hyperlipidemia and the abilities of *O. gratissimum* (SL) and *V. amygdalina* (BL) as well as their combined ability to attenuate hyperlipidemia by potentiating the activity of catalase was evaluated. The result revealed that hyperlipidemia inhibited the activity of catalase as an endogenous antioxidant enzyme. The extracts showed significant potencies by inducing the activity of catalase. The combination of

BL and SL gave a highly significant catalase activities as it attenuated hyperlipidemia (p<0.05). BL demonstrated least ability to potentiate the activity of catalase with and without hyperlipidemia.

Evaluation of the activity of superoxide dismutase (Figure. 2) revealed the distinction between the administrations of *O. gratissimum* (SL) and *V. amygdalina* (BL) as well as when combined for the amelioration of the disorder. The induction of hyperlipidemia by the high fat diet reduced the activity of superoxide dismutase (an endogenous antioxidant enzyme that catalyzes the breakdown of superoxide radical via dismutation to hydrogen peroxide and prevents the formation of hydroxyl radical). The administration of *O. gratissimum* (SL) and *V. amygdalina* (BL) as well as their combined ability did not give distinct significant difference, however the result revealed that they ameliorated hyperlipidemia by raising superoxide dismutase activity.

Figure 3 is the result for triglyceride concentration (mg/dl) in the serum. The result revealed that BL 14DT potentiated significantly higher triglyceride concentration than BL7DT. BL7DT and BL+SL7DT were partially significantly different, though BL+SL7DT group had higher TAG concentration. SL 14DT and SL7DT were not distinctly significant in relation. BL reduced TAG significantly more than SL, however there was no significant difference between BL14DT and SL7DT (p<0.05). Triglyceride in the heart as revealed by Figure. 3 showed no significant difference between BL7DT and BL14DT (p<0.05), there was no significant difference

between SL7DT, BL+SL7DT, BL+SL14DT but slight significant difference in SL14DT (p<0.05). TAG evaluation in the brain (Figure. 3) also showed a lower and significantly different values for BL14DT and BL7DT against SL7DT, SL14DT, BL+SL7DT and BL+SL14DT which are all partially or not significant relationship (p<0.05). Figure. 3 revealed the distribution of TAG in the hepatocytes. The results obtained for liver were closely related to the results obtained for serum, heart and brain. However, there was no significant different between BL7DT and BL14DT (p<0.05), meanwhile, SL14DT and SL7DT were slightly significantly different (p<0.05). Figure. 3 was the results for TAG concentration in the kidney, result revealed similar findings as brain, heart, serum and liver. Figure 3 further revealed that high fat intake increased the TAG in all the experimented tissues. A consistent reduction of TAG was observed in cases of treatment of *V. amygdalina* (BL) against hyperlipidemia by high fat diet after 7 and 14 days (HYPL+BL7DT and HYPL+BL14DT) in all the evaluated tissues. There was significant difference between HYPL+SL14DT (as well as HYPL+SL7DT) and HYPL+BL14DT (as well as HYPL+BL7DT) (p<0.05). The combined anti-hyperlipidemic effect of BL and SL revealed a reduced activity of *V. amygdalina* (BL) and *O. gratissimum* (SL) in reduction of TAG, thus there was a

significantly higher TAG deposition in all evaluated tissues (after high fat diet) upon treatment with BL+SL for 14 and 7 days duration (HYPL+BL+SL14DT and HYPL+BL+SL7DT respectively) (p<0.05). These results competed with the more effective BL administration against TAG concentration increase, caused by high fat diet (HYPL+BL7DT and HYPL+BL14DT). Also, HYPL+SL7DT and HYPL+SL14DT potentiated reduction in TAG concentration significantly, compared to HYPL+BL+SL14DT and HYPL+BL+SL7DT (p<0.05). Results revealed (Figure. 3) that serum had higher TAG concentration compared to other tissues.

Figure 4 revealed significantly lower total cholesterol (induced by high fat diet) by BL for all the evaluated tissues. There was slight significant difference between HYPL+BL7DT and HYPL+BL14DT which was observed in all the tissues except kidney, in which there was no significant difference. Results on Figures 4 revealed HYPL+SL7DT and HYPL+SL14DT groups as having slight significant difference in all the tissues except brain, in which no significant difference was analyzed (p<0.05). The effect of combination of *V. amygdalina* (BL) and *O. gratissimum* (SL) is the common reduction in the anti-hyperlipidemic activities of the individual leaf extract for all the tissues.
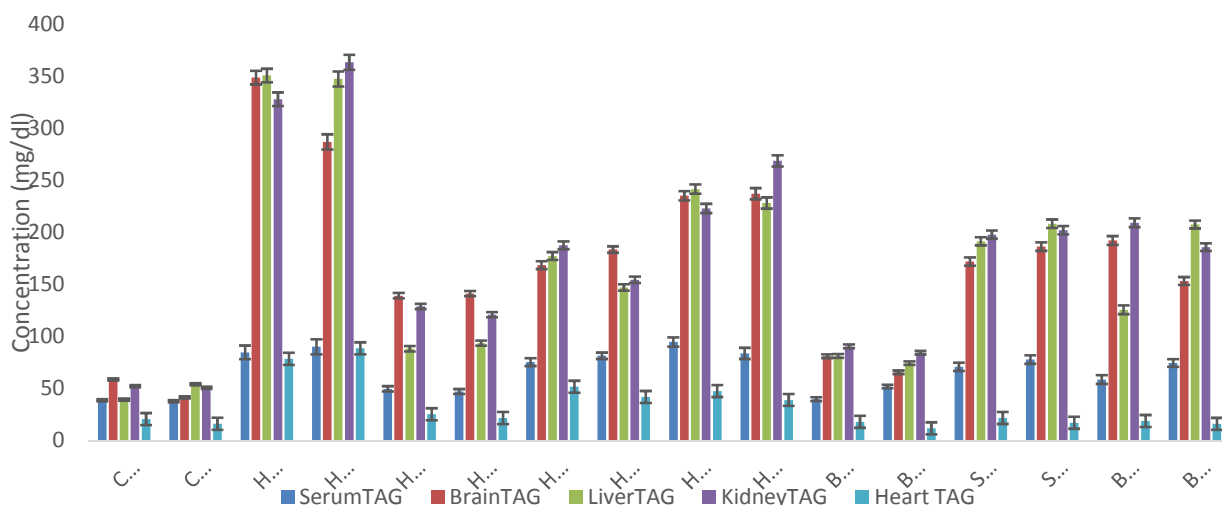

Figure 3: Alterations of Concentration of Triglycerides in Heart, Serum, Brain, Liver and Kidney by hyperlipidemia and antihyperlipidemic activities of extracts of the leaves of *V. amygdalina* and *O. gratissimum*. Each value represents mean (n=5).CNT 7DT-Control group after 7-day time; CNT 14DT-Control group after 14-day time; HYPL 7DT-Hyperlipidemic group without treatment for 7-day; HYPL+B.L 7DT- Hyperlipidemic group with *V. amygdalina* treatment for 7-day; HYPL+B.L 14DT- Hyperlipidemic group with *V. amygdalina* treatment for 14-day; HYPL+S.L 7DT- Hyperlipidemic group with *O. gratissimum* treatment for 7-day; HYPL+S.L 14DT- Hyperlipidemic group with *O. gratissimum* treatment for 14 days; HYPL+B.L+S.L. 7DT- Hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 7-day; HYPL+B.L+S.L. 14DT- Hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 14-day; B.L 7DT- Non-hyperlipidemic group with *V. amygdalina* treatment for 7-day; B.L 14DT- Non-hyperlipidemic group with *V. amygdalina* treatment for 14-day; S.L 7DT- Non-hyperlipidemic group with *O. gratissimum* treatment for 7-day; S.L 14DT- Non-hyperlipidemic group with *O. gratissimum* treatment for 14-day; B.L+S.L 7DT- Non-hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 7-day and B.L+S.L 14DT-Non-hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 14-day
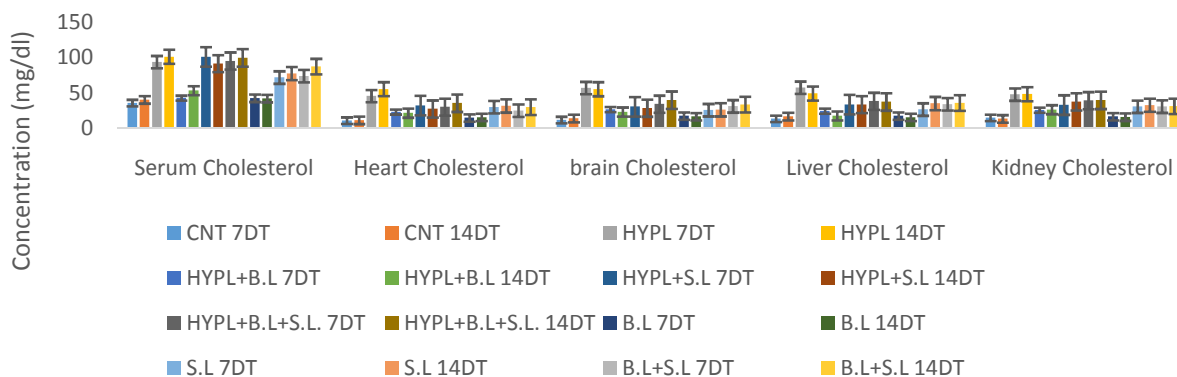
Figure 4: Alterations of Concentration of Total Cholesterol in Heart, Serum, Brain, Liver and Kidney by hyperlipidemia and antihyperlipidemic activities of extracts of the leaves of *V. amygdalina* and *O. gratissimum*. Each value represents mean (n=5). CNT 7DT-Control group after 7-day time; CNT 14DT-Control group after 14-day time; HYPL 7DT-Hyperlipidemic group without treatment for 7-day; HYPL+B.L 7DT- Hyperlipidemic group with *V. amygdalina* treatment for 7-day; HYPL+B.L 14DT- Hyperlipidemic group with *V. amygdalina* treatment for 14-day; HYPL+S.L 7DT- Hyperlipidemic group with *O. gratissimum* treatment for 7-day; HYPL+S.L 14DT- Hyperlipidemic group with *O. gratissimum* treatment for 14 days; HYPL+B.L+S.L. 7DT- Hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 7-day; HYPL+B.L+S.L. 14DT- Hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 14-day; B.L 7DT- Non-hyperlipidemic group with *V. amygdalina* treatment for 7-day; B.L 14DT- Non-hyperlipidemic group with *V. amygdalina* treatment for 14-day; S.L 7DT- Non-hyperlipidemic group with *O. gratissimum* treatment for 7-day; S.L 14DT- Non-hyperlipidemic group with *O. gratissimum* treatment for 14-day; B.L+S.L 7DT- Non-hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 7-day and B.L+S.L 14DT-Non-hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 14-day

The evaluation of HDL in serum was revealed in Figure. 5. *V. amygdalina* (BL) raised the concentration of HDL significantly and it also yielded a positive result as it ameliorated hyperlipidemia with respect to duration of administration. There was no significant difference in HDL concentration as revealed by the result for *O. gratissimum* (SL) administration with (HYPL+SL7DT and HYPL+SL14DT) or without (SL7DT and SL14DT) hyperlipidemia. Moreover there was no significant difference between HDL concentration for the administrations of *O. gratissimum* (SL) and when combined with *V. amygdalina* (BL) even in the amelioration of the disorder (p<0.05).
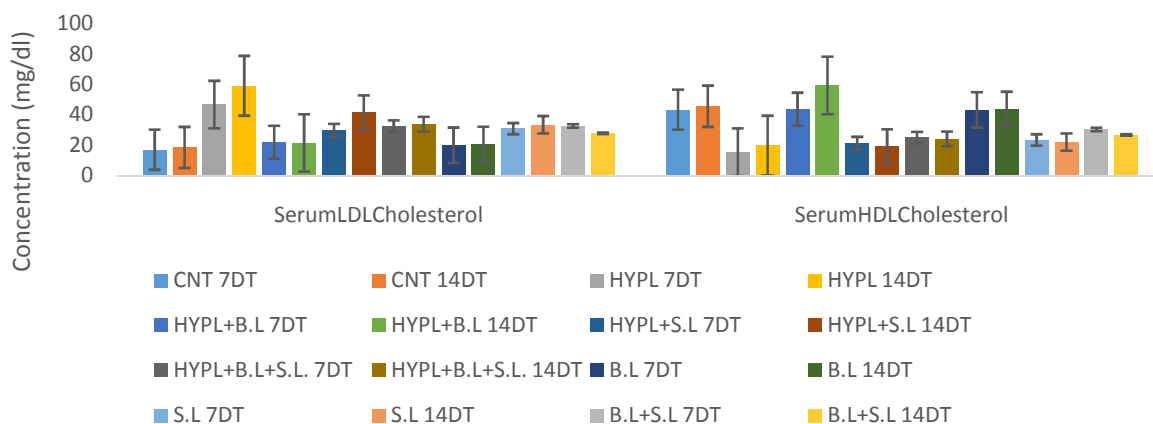

Figure 5: Alterations of Concentrations of HDL and LDL Cholesterols in Serum by hyperlipidemia and antihyperlipidemic activities of extracts of the leaves of *V. amygdalina* and *O. gratissimum*. Each value represents mean (n=5). CNT 7DT-Control group after 7-day time; CNT 14DT-Control group after 14-day time; HYPL 7DT-Hyperlipidemic group without treatment for 7-day; HYPL+B.L 7DT- Hyperlipidemic group with *V. amygdalina* treatment for 7-day; HYPL+B.L 14DT- Hyperlipidemic group with *V. amygdalina* treatment for 14-day; HYPL+S.L 7DT- Hyperlipidemic group with *O. gratissimum* treatment for 7-day; HYPL+S.L 14DT- Hyperlipidemic group with *O. gratissimum* treatment for 14 days; HYPL+B.L+S.L. 7DT- Hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 7-day; HYPL+B.L+S.L. 14DT- Hyperlipidemic group with *V. amygdalina* + *O. gratissimum* treatment for 14-day; B.L 7DT- Non-hyperlipidemic group with *V. amygdalina* treatment for 7-day; B.L 14DT- Non-hyperlipidemic group with *V. amygdalina* treatment for 14-day; S.L 7DT- Non-

hyperlipidemic group with *O. gratissimum* treatment for 7-day; S.L 14DT- Non-hyperlipidemic group with *O. gratissimum* treatment for 14-day; B.L+S.L 7DT- Non-hyperlipidemic group with *V. amygdalina + O. gratissimum* treatment for 7-day and B.L+S.L 14DT-Non-hyperlipidemic group with *V. amygdalina + O. gratissimum* treatment for 14-day

The effects of high fat diet on phospholipids of the serum, heart, brain, liver and kidney were revealed in Figures 6. The treatment with BL attenuated the hyperlipidemic effect significantly compared to SL and the combined effect of SL and BL. This result remained valid for all the phospholipid evaluations in the tissues (p<0.05).
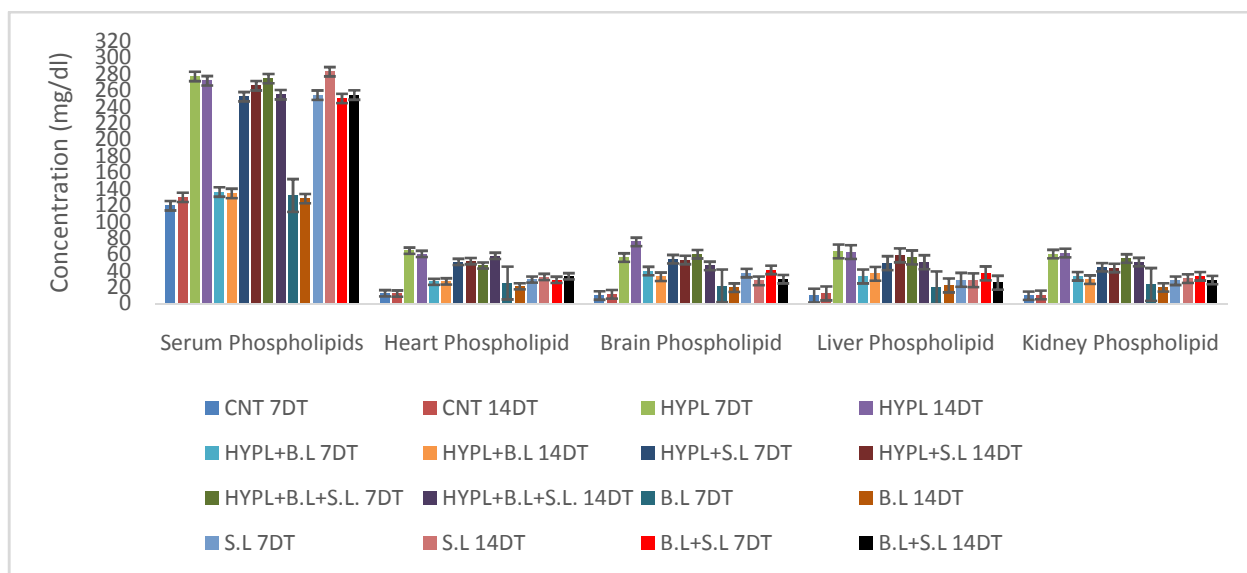

Figure 6: Alterations of Concentration of Phospholipids in Heart, Serum, Brain, Liver and Kidney by hyperlipidemia and antihyperlipidemic activities of extracts of the leaves of *V. amygdalina* and *O. gratissimum*. Each value represents mean (n=5). CNT 7DT-Control group after 7-day time; CNT 14DT-Control group after 14-day time; HYPL 7DT-Hyperlipidemic group without treatment for 7-day; HYPL+B.L 7DT- Hyperlipidemic group with *V. amygdalina* treatment for 7-day; HYPL+B.L 14DT- Hyperlipidemic group with *V. amygdalina* treatment for 14-day; HYPL+S.L 7DT- Hyperlipidemic group with *O. gratissimum* treatment for 7-day; HYPL+S.L 14DT- Hyperlipidemic group with *O. gratissimum* treatment for 14 days; HYPL+B.L+S.L. 7DT- Hyperlipidemic group with *V. amygdalina + O. gratissimum* treatment for 7-day; HYPL+B.L+S.L. 14DT- Hyperlipidemic group with *V. amygdalina + O. gratissimum* treatment for 14-day; B.L 7DT- Non-hyperlipidemic group with *V. amygdalina* treatment for 7-day; B.L 14DT- Non-hyperlipidemic group with *V. amygdalina* treatment for 14-day; S.L 7DT- Non-hyperlipidemic group with *O. gratissimum* treatment for 7-day; S.L 14DT- Non-hyperlipidemic group with *O. gratissimum* treatment for 14-day; B.L+S.L 7DT- Non-hyperlipidemic group with *V. amygdalina + O. gratissimum* treatment for 7-day and B.L+S.L 14DT-Non-hyperlipidemic group with *V. amygdalina + O. gratissimum* treatment for 14-day.

Discussion

The plants extracts were each tested for their antioxidant properties using different standard antioxidant protocols. The total antioxidant activities of *O. gratissimum* and *V. amygdalina* according to table 1 revealed a significantly lower total antioxidant activity of *O. gratissimum* when compared with *V. amygdalina* . Also, the reducing power of the aqueous leaf extracts of *V. amygdalina* potent higher antioxidant activity than *O. gratissimum*. Reducing power of medicinal plants is an important indices for defining their antioxidant ability. Reducing power is considered a defense mechanism which is related to the ability of the antioxidant agents to transfer electron or hydrogen atom to oxidants or free radicals [23]. Antioxidants are functional reducing agents with the ability to protonate oxidizing agents like DPPH. The aqueous extracts of leaf of *V. amygdalina* and *O. gratissimum* scavenged DPPH radical although the former has a better potential in this effect. Similar inference was achieved as the combination of the leaf extract countered the high activity of *V. amygdalina* which scavenged DPPH radical by protonation when combined with *O. gratissimum*. *V. amygdalina* demonstrated higher ABTS radical scavenging activity than *O. gratissimum* were significantly competitive with that of trolox. Since all extracts exhibited scavenging activities against DPPH and ABTS radicals, this further indicated the capabilities of the extracts to scavenge different free radicals in different systems, indicating that they could be useful therapeutic agents for treating radical-induced pathological damage. The ABTS radical scavenging activity is peculiar with the leaves extracts of *V. amygdalina* and *O. gratissimum*. The hydroxyl radical is the most reactive oxygen species, and it induces severe

damage in adjacent biomolecules [24]. Studies had shown that the reactive oxygen species of low reactivity could be converted to a highly reactive species [25]. Reaction of hydrogen peroxide (H_2O_2) with low valence forms of the transition metal ions; iron (Fe^{2+}) and Copper(Cu^{2+}) ion lead to the formation of ·OH (Fenton reaction) or species of comparable reactivity such as Fe^{2+} (Ferryl ion) or $Cu(OH)^{2+}$ a copper III complex. The ability of these leaves extracts to chelate this reactive metal iron was an indication of their antioxidant activity which defined the explanation for the protection of biomolecules such as nucleic acid, protein and lipids against oxidative damage and anti-inflammatory activity. *V. amygdalina* possessed higher metal chelating activity than *O. gratissimum.* The aqueous leaf extracts of *V. amygdalina* scavenged hydroxyl radical more effectively than *O. gratissimum.*

Generally, the hyperlipidemias are of interest to the physician in the context of risk factors for ischaemic heart disease (IHD) and peripheral vascular disease [26]. The first step in diagnosis of hyper- and hypolipoproteinaemias is to define the lipoprotein pattern by chemical analysis of the plasma lipids and lipoproteins [26]. Abundant evidence has accumulated relating the concentrations of lipids (total cholesterol and triglycerides) and their associated blood transporting lipoproteins (HDL-C, LDL-C, VLDLC) with the occurrence of atherosclerosis in general and coronary artery disease (CAD) in particular [27]. The strong association between the risk of coronary artery diseases (CAD), high levels of LDL-C and low levels of HDL-C has been well established [28] and different plant extracts had been used in past research works to effect lipidomic effect. [19] described the lipid lowering ability of *V. amygdalina* in rats fed with high cholesterol diet for a period of nine weeks. Thus, this work aimed of at comparing the lipid lowering capacities of the leaves of *V. amygdalina* and *O. gratissimum.* The results in this study remained consistent with those of [19] as it can be inferred that *V. amyygdalina* leaves extracts modulated the induced dyslipidemia in a beneficial manner although the effect was lower when compared with *O. gratissimum* leaves extract.The crucial risk factor for CVD includes a low level of HDL-cholesterol [29]. The association between a low level of HDL-cholesterol and an increased risk of CVD has been well established through epidemiological and clinical studies [30]. Since low level of HDL cholesterol plays a direct role in the atherogenic process, therapeutic intervention to raise HDL-cholesterol together with other risk factors is widely encouraged [19]. In this study, the evaluation of HDL-c revealed that *O. gratissimum* and *V. amygdalina* increased HDL-c, indicating their promising potentiating roles for HDL cholesterol against CVD. *O. gratissimum* had lesser HDL-c promoting activity compared to *V. amygdalina.* The protective roles of HDL cholesterol from CVD have been suggested to occur in various ways [31]. HDL exerts part of its anti-atherogenic effect by counteracting LDL oxidation and, recent studies also showed that HDL promotes the reverse cholesterol transport pathway, by inducing an efflux of excess accumulated cellular cholesterol and prevents the generation of an oxidatively

modified LDL [32]. Furthermore, HDL inhibits the oxidation of LDL by transition metal ions, but also prevents 12-lipooxygenase-mediated formation of lipid hydroperoxides [31]. On the basis of these results, *O. gratissimum* and *V. amygdalina* could probably be promoting the anti-atherogenic role of HDL-c through the inhibition of lipids oxidation (due to its anti-lipoperoxidative effect observed in this study). Elevated LDL resulted in deposition along the blood vessel, thus becoming the inducer of atherosclerosis lesion. These results showed that *V. amygdalina* elicited more beneficial effects than *O. gratissimum* by lowering the plasma levels of LDL-c of the treated rats and suggests its important role in the ameliorating of atherosclerosic diseases.

Phospholipids (PLs) contents was higher in VA treated organs than OG treated ones although both extracts significantly (P<0.05) increased PLs in both dyslipidemic organs and the healthy ones. PLs have a major impact in several diseases; apparently they were shown to reduce side effects of some drugs [34] Precisely, these effects can partially be explained by the fact that PL are highly effective in delivering their fatty acid (FA) residues for incorporation into the membranes of cells involved in different diseases, e.g. immune or cancer cells. [33]The altered membrane composition is assumed to have effects on the activity of membrane proteins (e.g. receptors) by affecting the microstructure of membranes and, therefore, the characteristics of the cellular membrane, e.g. of lipid rafts, or by influencing the biosynthesis of FA derived lipid second messengers [33]. However, since the FAs originally bound to the applied PLs are increased in the cellular membrane after their consumption or supplementation, the FA composition of the PL and thus the type of PL is crucial for its effect.

Conclusion

The research study had been able to establish that aqueous leaves extracts of *V. amygdalina, O. gratissimum* and the association had highly potent secondary and primary antioxidant which had demonstrated metal chelating activity as well as chain breaking potency respectively. The study further established that *V. amygdalina* had the higher obvious invitro antioxidant activities compared to *O. gratissimum.* The lipid modulating activities of *V. amygdalina* and *O. gratissimum* suggested that both plant extracts confers beneficial effects on the lipidomic status of albino rats, although *V. amygdalina* had higher antidyslipidemic ability manifested in its ability to ameliorate the induced hyperlipidemia than *O. gratissimum.* The dietary effects of these leaves were studied and the high impact of *V. amygdalina* was established independently and with respect to *O. gratissimum.*

Conflicts of interests

The authors declare no conflicts of interests.

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