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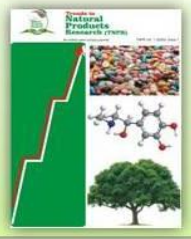

Antihyperlipidemic and Antioxidant Activities of Catechin-rich Extract and Fractions of *Phaseolus vulgaris*, A Traditional Therapy for Overweight Diabetes Subjects

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Abstract: *Phaseolus vulgaris* is used in ethnomedicine for the management of overweight and cardiovascular disorders with claims of efficacy. This motivated this study in which the antihyperlipidemic activities of the extracts of *P. vulgaris* and its antioxidant activities were evaluated. Methanol/methylene chloride (1:1) extract was screened and further partitioned into n-hexane, ethyl acetate and butanol solvent fractions. HPLC "fingerprinting" technique showed the abundance of catechin in the crude extract. Oral median lethal dose (LD_{50}) was estimated to be greater than 5000 mg/kg. Short term treatment with the crude extract and ethyl acetate fraction significantly ($p < 0.05$) lowered the levels of serum triglyceride, total cholesterol, LDL-C and VLDL-C in hyperlipidemic condition induced in rats with Triton WR-1339, Triton X-100 and high fat diet. Similarly, sub-acute treatment of the animals with crude extract and ethyl acetate fractions produced dose-dependent and significant ($p < 0.05$) decrease in serum triglyceride, total cholesterol and VLDL-C levels in high fat hyperlipidemic model. Antioxidant activities of the extract and fractions were determined *in vitro* using DPPH assay while liver malondialdehyde and serum antioxidant enzyme activities were used to assess the *in vivo* antioxidant potentials in CCl_4-intoxicated rats. DPPH median inhibitory concentrations (IC_{50}) of the extract and fractions ranged from 79.84-98.49 µg/ml. The extract and its fractions produced significant ($p < 0.05$) inhibition of lipid peroxidation and significant ($p < 0.05$) higher levels of catalase and glutathione peroxidase enzyme activities. This investigation showed that the extract and fractions of *Phaseolus vulgaris* possess some anti-hyperlipidemic and

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antioxidant activities that are relevant to its use in traditional medicine.

INTRODUCTION

Hyperlipidemia is an abnormal lipid metabolism associated with the elevated levels of cholesterol, triglyceride and their associated lipoproteins (low-density and very-low-density lipoproteins).This condition is most commonly associated with high-fat diets, sedentary lifestyle, obesity, diabetes and genetic abnormalities (Rosini *et al.,* 2012). Hyperlipidemia is of clinical relevance because of its association with an increased risk of cardiovascular diseases. Increased plasma levels of total cholesterol and low-density lipoproteins have been demonstrated by multiple epidemiologic studies to be strongly and directly related to a greater incidence of coronary heart disease (Millan *et al.,* 2009). Elevated plasma triglycerides and very-low-density lipoproteins are also directly associated with the risk of atherosclerotic heart disease (Upadhyay, 2015). High density lipoprotein on the contrary was found to be a protective factor against the development of cardiovascular diseases; however, decreased levels constitute a risk factor (Bruckert and Hansel, 2007).

Despite many health interventions, cardiovascular diseases (CVD) remain a significant health burden and a leading cause of mortality in many countries (Kolandaiveln *et al.,* 2014).Among the numerous contributors to the pathogenesis of this disease condition, hyperlipidemia stands as a major risk factor (Nelson, 2013).Chemotherapeutic uses for the control of lipid concentration in CVD are however associated with inadequate efficacies and adverse effects. Statin drugs like atovastatin, lovastatin and simvastatin use in lowering plasma cholesterol levels in cardiovascular patients are not devoid of obvious side effects on muscular system, hepatic and renal functions (Barakat *et al.,* 2013).These challenges have led to continual interest in the search for more effective drugs with less adverse effects.

In hyperlipidemic state, formation of reactive oxygen species (ROS) is of pivotal pathological importance due to the formation of oxidized lipids. ROS induced oxidation of low density lipoprotein (LDL) stimulates further formation and release of ROS with enhanced adhesion molecules expression, platelet activity and other pro-atherogenic effects in the vasculature leading to CVD (Ellulu *et al.,* 2016).The therapeutic and experimental efficacy of various antioxidant substances in the management of CVD further supports the role of augmented ROS formation in these disease states (Jain *et al.,* 2015).

Phaseolus vulgaris L. (Leguminosae) commonly known as kidney bean is used in traditional medicine mainly for treatment of diabetes and overweight. In streptozotocin-induced diabetic rats, the aqueous extract was shown to suppress diabetes induced hyperlipidemia with elevation of circulating antioxidants (Venkateswaran, 2002). Its anti-inflammatory (Oomah *et al.,* 2010), antimutagenic (Frassinetti *et al.,* 2015) and antimicrobial activities (Kumar *et al.,* 2014) have also been reported. This study evaluated the antihyperlipidemic activities for short, medium and long-term treatment, as well as the antioxidants potentials of the extract and various solvent fractions of *Phaseolus vulgaris*.

MATERIALS AND METHODS

Plant Materials

Fresh fruit of *Phaseolus vulgaris* L. was obtained from Obollo-Afor in Nsukka Local Government Area, Enugu State, Nigeria and authenticated by Mr. A. Ozioko, a taxonomist with the Bio-resources Development and Conservation Programme (BDCP) center, Nsukka, Enugu State, Nigeria.

Animals

Adult male Swiss albino mice (20-25 g) and Wistar albino rats (150-200 g) were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. Animals were housed under standard conditions and fed with standard pellets (Guinea Feeds Nigeria Ltd) and water *ad libitum*. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals (Pub No: 85-23 Revised 1985).

Extraction and Fractionation

The plant materials were air dried and pulverized. A 500 g of the powdered extract was cold macerated for 48 h in 1250 ml of a mixture of methanol and methylene chloride in the ratio 1:1 to obtain the crude extract. Two-third of the extract was partitioned successively with solvents of increasing order of polarity using liquid-liquid chromatographic technique to obtain n-hexane, ethylacetate and n-butanol fractions. The extract and fractions were concentrated at 40 °C using rotary evaporator fitted with a vacuum pump.

Phytochemical Screening

Qualitative determination of the phyto-constituents in the extract and fractions were carried out using the standard procedures as described by Sofowora (1993) and Trease and Evans (2002).

HPLC-fingerprinting

HPLC analysis was performed on the samples with a Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S, Dionex Softron GmbH, Germany). Detection was at 235 nm. The separation column (125 x 4 mm; length x internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Germany), and a linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent.

Acute Toxicity (LD_{50}) Study

The acute toxicity (LD_{50}) study was carried on the crude extract of *Phaleosus vulgaris* L. using Lorke's method (Lorke, 1983). Animals (Wistar albino rats), grouped into three groups of three animals each received oral doses of 10, 100 and 1000 mg/kg in phase I and were observed for 24 h. As determined by the outcome of the first stage, doses of 1600, 2500, 3900 and 5000 mg/kg were given respectively to four different mice each in phase II. The median lethal dose (LD_{50}) was estimated as the geometrical mean of the least lethal dose and highest non-lethal dose after observing the animals for 24 h for obvious signs of acute intoxication or mortality.

Antihyperlidemic Studies

Acute, subacute and chronic study to determine the lipid lowering effects of extract and fractions of *Phaseolus vulgaris* was carried out. Fifty rats randomly divided into 10 groups of five rats each were used. Groups 1 and 2 received the extract, 3 and 4 n-hexane fraction, 5 and 6 ethyl acetate fraction, 7 and 8 butanol fraction. The extract and fractions were administered at the doses of 100 and 200 mg/kg respectively. Group 9 served as the positive control and received 10 mg/kg of atorvastatin while Group 10 (negative control) received 10 ml/kg of vehicle. All treatments were done orally.

Short-term Administration (acute) Study

Each group was given the various doses of the test drug or the control, followed an hour later with intra-peritoneal administration of 300 mg/kg of Triton WR-1339 (Korolenko*et al.*, 2010). Blood was drawn from the retro-orbital plexus of the rats for biochemical analysis 24 h after treatment. Biochemical analysis of the sera samples was done using Randox® lipid profile commercial kit.

Medium-term Administration (sub-acute) Study

In this model, hyperlipidemia was induced using 100 mg Trition-X 100/kg to the rats in each group. After 72 h of trition administration, the extract and fractions were administered daily for 7 days. Blood sample was drawn on the 8th day from each rat through retro-orbital plexus for biochemical analysis (Gundamaraju *et al*., 2014).

Long-term (chronic) Study

For chronic hyperlipidemia, high fat diet was used to induce hyperlipidemia in this investigation (Thirumalai *et al.*, 2014). The high fat diet comprised of chow enriched with high calorie and 1 % cholesterol. High fat diet induced hyperlipidemia is established model of chronic hyperlipidemia (Chandratre *et al.*, 2011). Baseline lipid profile was determined before the introduction of high fat diet. Seven days after feeding the rats on high, the lipid profile was determined again to ascertain successful induction of hyperlipidemia. For 21 days, the hyperlipidemic rats were treated with the extract and fractions or as control while they were still maintained on high fat diet. Blood samples were drawn on the 22nd day for the determination of the effect of the extract and fractions on the lipid profile.

***In Vitro* Antioxidant Study**

DPPH Scavenging Activity

The free radical scavenging activity of the test substances was determined using previously reported procedure (Obiagwu *et al*., 2014). Freshly prepared DPPH was incubated at room temperature with serial concentrations of the extract and fractions (25, 50, 100, 200 and 400 µg/ml) in duplicate. The same serial dilutions of ascorbic acid were used as standard. After 30 min, the absorbance was measured at 517 nm. Percentage DPPH scavenging activity was calculated thus:

$$\% \text{ Inhibition of free radical} = \left[\frac{A_0 - A_t}{A_0}\right] \times 100$$

Where A_0 is the absorbance of the control, and A_t is the absorbance of the test/standard. The EC_{50} was determined from a plot of percentage scavenging potentials against concentration.

***In Vivo* Antioxidant Study**

Fifty-five rats were used for this study and were similarly grouped as described for the antihyperlipidemic study. Ascorbic acid was used as the standard treatment. The animals were given intraperitoneal administration of 1 ml/kg of CCl_4 for 2 days followed by oral administration of the extract and fractions for 7 days. After an overnight fast, blood samples were drawn from the retro-orbital plexus for the determination of antioxidant enzyme activities. The animals were then sacrificed thereafter and the liver harvested for lipid peroxidation assay.

Estimation of Catalase Activity

The rate of breakdown of hydrogen peroxide was used to monitor catalase enzyme activity according to the method of Aebi (1983).The reaction mixture was composed of 2.5 ml of phosphate buffer, 2 ml of hydrogen peroxide and 0.5 ml of sample. To 1 ml portion of the mixture, 2 ml of dichromate acetic acid reagent was added. The rate reaction was monitored using UV-spectrophotometer at 240 nm for 3 min at a minute interval.

Estimation of Lipid Peroxidation

Malondialdehyde (MDA) - an aldehyde product of lipid peroxidation was used as an index of lipid peroxidation. The reaction mixture was composed of 0.1 ml of liver tissue homogenate and 2 ml of TBA-TCA-HCl reagent (1:1:1 ratio, 0.37 % TBA, 0.25 N HCl, 10 % TCA) which was incubated at 95° C for 40 min. Sodium dodecyl sulphate (SDS, 20 %, 0.1 ml) was added and the absorbance was taken at 532 nm against a blank (Wallin *et al.,* 1993).

Estimation of Glutathione Peroxidase

Glutathione peroxidase (GPx) activity was determined using the method of Agergaard and Jensen (1982). The reaction mixture contains 0.1 ml of liver tissue homogenate, 3 ml of phosphate buffer, 0.05 ml of Gluaiacol, and 0.03 ml of hydrogen peroxide. The absorbance was taken at 436 nm for 2 min at 30 sec interval.

Statistical Analysis

All data obtained were analysed using Graph pad Prism™ and expressed as Mean ± SEM Differences between group means were compared using one way ANOVA followed by Dunnet's post-hoc test and $p < 0.05$ were considered significantly different.

RESULTS

Phytochemical Constituents of the Extract and Fractions

Extraction of pulverized dried fruits of *P. vulgaris* yielded 58.6 g (11.72 %) extract. Two-thirds of this extract was found to be partitioned into n-hexane 11.24 g (28.77 %), ethyl acetate 13.12 g (33.58 %) and butanol 14.71 g (37.65 %) fractions (Table 1). Saponins, tannins and flavonoids were found to be abundant in the extract while ethyl acetate fraction showed abundance of saponins and flavonoids (Table 1). Saponins were also found to be abundantly distributed in the butanol fraction.

HPLC fingerprint

HPLC fingerprint of the extract showed 4 major peaks (Figure 1) numbered A – D with retention times at 26.25, 32.59, 33.21 and 34.69 min respectively. Peak A corresponds to an isoflavone–daidzin while peaks B (the major compounds) and C correspond to catechin based on their characteristic UV curves. No spectrum hit was found for peak D (Figure 1).

Acute Toxicity (LD_{50})

Single daily dosing of the extract up to 5000 mg/kg was found not to cause mortality in the animals. During the 24 h post administration monitoring of the animals, no obvious signs of toxicity were seen in all treatment groups. The LD_{50} of the extracts was therefore estimated to be greater than 5000 mg /kg body weight.

Acute Antihyperlipidemic Effects

The result of the effect of the extract and fractions on acute hyperlipidemia induced by Triton WR-1339 is as stated in Table 2. The vehicle treated group was characterized by marked elevation in total cholesterol, triglyceride, LDL-C and VLDL-C with decreased levels of DHL-C. Serum triglycerides, total cholesterol, LDL-C, VLDL-C were found to decrease significantly ($p < 0.05$) in a dose dependent order after pre-treatment with the crude extract, hexane and ethylacetate fractions compared with the vehicle treated control group. Significant ($p < 0.05$) elevations in HDL-C were also recorded for the extract and these fractions. The activities recorded by ethyl acetate fraction on these lipid parameters were similar to that of atorvastatin (reference standard). Butanol fraction only showed significant ($p < 0.05$) reduction for triglycerides and VLDL-C.

Sub-acute Antihyperlipidemic Effects

Sub-acute treatment with the extract, ethyl acetate and butanol fractions produced significant ($P < 0.05$) decrease in serum triglycerides, total cholesterol and VLDL-C levels (Table 3).These activities were dose dependent. The extracts and fractions at 200 mg/kg also showed significant ($p < 0.05$) reduction in LDL-C without corresponding significant increase in serum HDL-C.

Chronic Antihyperlipidemic Effects

Though the extract did not significantly ($p > 0.05$) reduce body weight of high fat fed animals, significant ($p < 0.05$) effects on body weights were however evident in the fraction treated groups especially in butanol fraction treated group (Table 4). The extract, ethyl acetate and butanol fractions significantly ($p < 0.05$) decreased serum

triglycerides, total cholesterol, LDL-C and VLDL-C in the chronic high fat induced hyperlipidemic study (Table 5). Significant ($p < 0.05$) increase in HDL-C was also recorded in the extract, n-hexane and butanol fractions treated groups.

Table I: Phytochemical Composition of the Extract and Fractions

Phytocompounds	Extract	N-hexane F.	Ethylacetate F.	Butanol F.
Alkaloids	+	+	+	-
Saponins	+++	++	+++	+++
Proteins	-	-	-	-
Tannins	+++	++	+++	++
Steroids	+	+	+	+
Terpenoids	+	-	-	+
Resins	-	-	-	-
Carbohydrates	+	++	++	
Flavonoids	+++	++	+++	++
Reducing Sugar	++	++	+	+

- Absent, + small amount, ++ moderately high, +++ very high

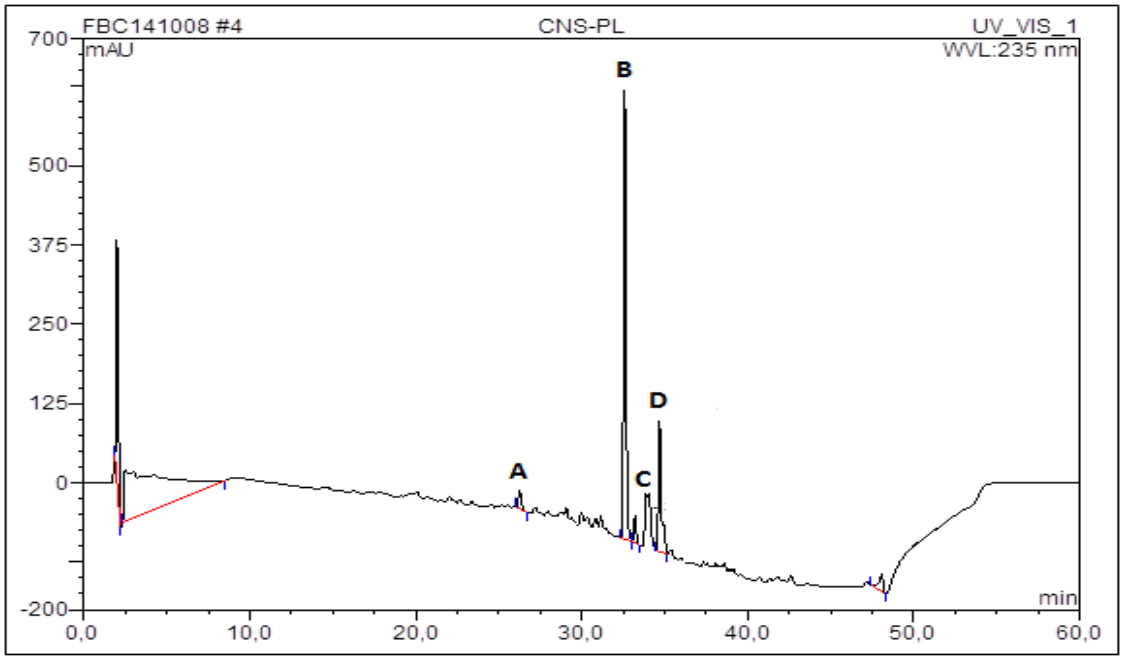

Figure I: HPLC fingerprint of *P. vulgaris* extract
A = Daidzin (Rt – 26.25 min), B = Catechin (Rt – 32.59 min), C = Catechin (Rt – 33.21 min), D = no spectral hit found (Rt – 34.69).

Table 2: Acute Effect of the Extract and Fractions on Lipid Parameters

Treatment	Dose (mg/kg)	TC (mg/dl)	TRIGS (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	AI
Extract	100	141.5±9.9	148.3±5.6	48.2±3.6	63.6±9.5*	29.7±1.1	0.49±0.04
	200	131.0±7.8*	139.7±7.7	51.5±1.9*	47.9±3.2*	27.9±1.5	0.43±0.03*
N-hexane F.	100	153.7±6.7	154.8±3.9	37.4±3.6	85.3±5.2	30.9±0.8	0.62±0.04
	200	132.5±8.1*	124.2±7.8*	58.0±2.9*	49.0±7.5*	24.8±1.6*	0.33±0.04*
E. acetate F.	100	140.4±10.9	148.3±5.8	56.5±3.5*	54.1±7.9*	29.7±1.2	0.42±0.02*
	200	128.2±5.4*	133.2±5.5*	58.3±4.5*	43.2±6.3*	26.7±1.1*	0.36±0.03*
Butanol F.	100	166.3±6.3	145.1±4.1	39.6±3.1	97.7±5.1	29.0±0.8	0.57±0.03
	200	151.5±8.7	127.1±5.7*	38.5±4.7	87.2±11.4	25.4±1.1*	0.53±0.08
Atorvastatin	10	128.9±6.7*	131.0±5.1*	59.4±2.3*	45.8±7.8*	26.2±1.0*	0.34±0.03*
Vehicle (5% Tween 20)	10 ml/kg	164.5±7.3	158.4±7.9	40.3±3.4	92.7±5.6	31.7±1.6	0.60±0.03

* $p < 0.05$ compared with vehicle treated group. F = fraction, TC = total cholesterol, TRIGS = triglyceride, HDL-C = high density lipoprotein – cholesterol, LDL-C = low density lipoprotein – cholesterol, VLDL-C = very low density lipoprotein – cholesterol, AI = Atherogenic index

Table 3: Sub-acute Effect of the Extract and Fractions on Lipid Parameters

Treatment	Dose (mg/kg)	TC (mg/dl)	TRIGS (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	AI
Extract	100	122.8±1.1	92.5±2.2	50.8±2.2	53.6±1.8	18.5±0.4	0.24±0.02
	200	119.9±0.9*	91.4±1.6*	52.6±2.6	46.0±3.2*	18.3±0.3*	0.24±0.02
N-hexane F.	100	125.9±1.4	98.6±3.9	47.9±6.8	56.9±6.1	19.7±0.8	0.33±0.09
	200	117.7±2.5	93.6±3.0	51.1±5.6	43.3±3.9*	19.3±0.8	0.27±0.09
E. acetate F.	100	120.1±6.3	98.3±3.5	49.3±2.9	51.7±9.0	19.7±0.7	0.30±0.02
	200	112.7±4.2*	99.0±3.0	55.4±2.1	40.4±6.7*	19.8±0.6	0.25±0.01
Butanol F.	100	121.3±3.2	95.8±5.1	45.7±2.6	62.5±9.1	19.2±1.0	0.32±0.02
	200	114.5±3.5*	82.8±0.8*	54.2±4.5	39.6±5.9*	16.6±0.4*	0.24±0.08
Atorvastatin	10	108.3±2.7*	84.2±1.3*	56.5±2.5*	34.9±5.6*	16.9±0.3*	0.17±0.03
Vehicle (5% Tween 20)	10 ml/kg	129.2±2.2	100.1±3.1	48.2±2.5	61.0±4.0	20.0±0.6	0.32±0.03

* $p < 0.05$ compared with vehicle treated group. F = fraction, TC = total cholesterol, TRIGS = triglyceride, HDL-C = high density lipoprotein – cholesterol, LDL-C = low density lipoprotein – cholesterol, VLDL-C = very low density lipoprotein – cholesterol, AI = Atherogenic index

Table 4: Chronic Effect of the Extract and Fractions on Body Weight

Treatment	Dose (mg/kg)	Basal body weight (g)	Post-treatment body weight (g)	Percentage reduction (%)
Extract	100	152.00±9.48	177.80±9.84	12.15
	200	149.00±8.80	170.60±8.69	15.71
N-hexane F.	100	145.40±9.16	170.20±7.90	15.91
	200	146.60±8.81	165.60±9.17*	18.18
Ethyl acetate F.	100	151.80±9.68	168.80±10.48	16.60
	200	147.80±9.07	164.80±6.80*	18.58
Butanol F.	100	145.40±8.82	163.40±8.17*	19.27
	200	150.00±6.39	165.00±7.01*	18.48
Atorvastatin	10	149.00±8.44	159.00±8.39*	21.44
Vehicle (5% Tween 20)	10 ml/kg	146.60±8.22	202.40±11.12	-

* $p < 0.05$ compared with vehicle treated group. Percentage reduction was calculated relative to vehicle treated group.

Table 5: Chronic Effect of the Extract and Fractions on lipid Parameters

Treatment	Dose (mg/kg)	TC (mg/dl)	TRIGS (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	AI
Extract	100	161.6±4.4	137.5±3.7	67.0±0.7	67.2±4.3*	27.5±0.8	0.32±0.02
	200	156.6±5.7*	133.2±3.7	67.7±1.9	62.3±4.6*	26.6±0.6	0.30±0.02*
N-hexane F.	100	166.0±2.2	147.9±3.9	60.1±1.5	76.3±2.0	29.6±0.8	0.44±0.09
	200	162.7±3.0	131.0±3.5	68.0±0.7*	64.4±4.1*	26.6±0.8	0.38±0.09
E. acetate F.	100	153.8±3.6*	136.1±2.3	61.6±3.4	65.5±5.6*	27.2±0.5	0.40±0.02
	200	158.0±2.2*	124.9±1.9*	53.6±0.7	79.6±2.0	25.0±0.4*	0.47±0.01
Butanol F.	100	161.3±3.0*	136.7±1.9	56.5±0.9	77.4±3.1	27.3±0.4	0.46±0.02
	200	159.7±2.2*	124.6±2.6*	65.2±1.0*	69.7±3.8*	24.9±0.5*	0.39±0.08
Atorvastatin	10	151.2±2.4*	123.5±1.9*	68.4±2.1	58.1±3.6*	24.7±0.4	0.25±0.01*
Vehicle (5% Tween 20)	10	173.1±2.9	138.6±1.8	58.3±1.4	87.1±3.5	27.7±0.4	0.38±0.01

* $p < 0.05$ compared with vehicle treated group. F = fraction, TC = total cholesterol, TRIGS = triglyceride, HDL-C = high density lipoprotein – cholesterol, LDL-C = low density lipoprotein – cholesterol, VLDL-C = very low density lipoprotein – cholesterol, AI = Atherogenic index

***In vitro* Antioxidant Scavenging Activity**

DPPH scavenging activity of the extract and fractions of *P. vulgaris* showed a concentration dependent activity (Figure 2). The extract gave IC_{50} of 98.49 µg/ml while the n-hexane, ethyl acetate and butanol fractions produced IC_{50}s of 93.69, 79.84 and 87.36 µg/ml, respectively. Ascorbic acid (the reference standard) showed IC_{50} 49.70 µg/ml.

The effect of the extract and fractions on serum antioxidant activities and liver lipid peroxidation is shown in Table 6 below. At 100 mg/kg, the extract showed significant ($p < 0.05$) increase in catalase enzyme activity similar to ascorbic acid. Significant ($p < 0.05$) increase in serum glutathione peroxidase was recorded for the fractions at all tested doses while only 200 mg/kg of butanol fraction significantly ($p < 0.05$) inhibited liver lipid peroxidation compared with the vehicle treated group.

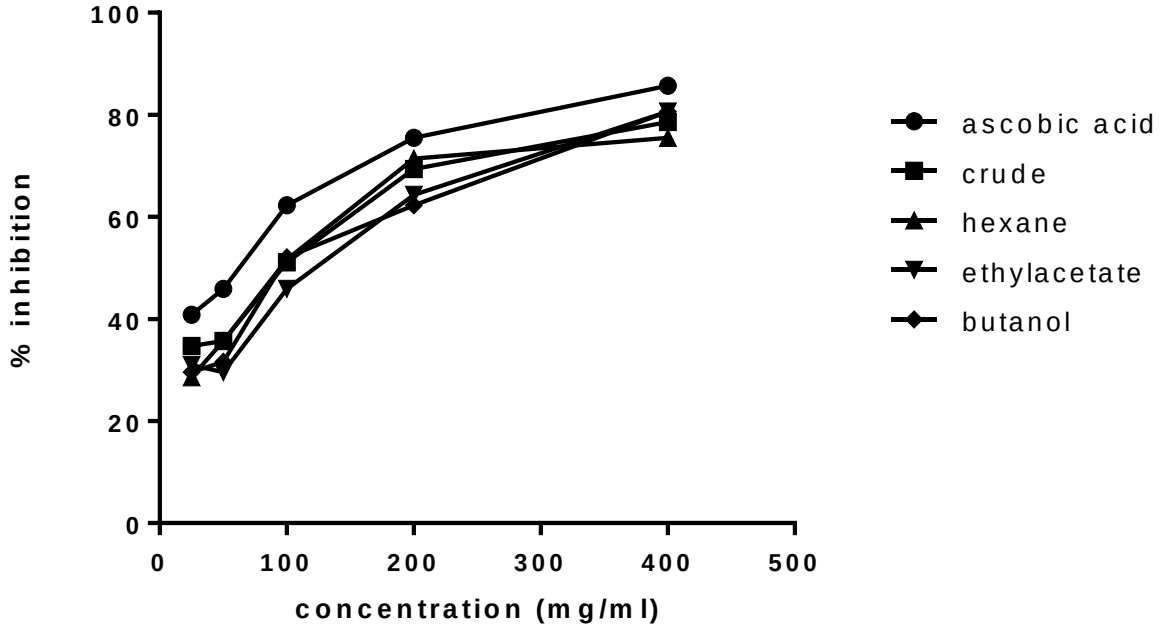

Figure 2: DPPH scavenging activities of the extract and fractions of *P. vulgaris*

Table 6: Effects of the Extract and Fractions on Antioxidant Enzymes and Lipid Peroxidation

Treatment	Dose (mg/kg)	Catalase (IU/L)	GPx (IU/L)	MDAµM/g liver tissue
Extract	100	2.09±1.68*	0.43±0.04	2.44±0.57
	200	1.16±0.14	0.34±0.02	1.55±0.12
N-hexane F.	100	0.68±0.16	1.25±0.12*	1.57±0.11
	200	0.82±0.27	1.40±0.21*	1.54±0.42
E. acetate F.	100	0.62±0.09	1.13±0.21*	1.03±0.36
	200	0.40±0.01	1.20±0.09*	1.48±0.06
Butanol F.	100	0.75±0.17	1.68±0.06*	1.25±0.22
	200	0.89±0.22	1.21±0.17*	0.52±0.10*
Ascorbic acid	100	2.32±0.73*	0.99±0.15*	2.18±0.45
Vehicle(5% Tween 20)	5 ml/kg	0.65± 0.41	0.40±0.04	1.40±0.10

All values expressed as Mean ± SEM, where n=5. $p < 0.05$ compared with the vehicle treated group.

DISCUSSION

Disturbances occurring in lipid metabolism have been perceived as a contributive factor of cardiovascular diseases (Nelson, 2013). Substances that intervene in dysfunctional lipid metabolic pathways are expected to reduce the incidence and mortality associated with these diseases. Discovery of improved, effective and safer antihyperlipidemic drugs for the prevention and management of cardiovascular diseases will overcome the challenges and shortcomings associated with existing antihyperlipidemic drugs, such as limited efficacies, severe adverse effects and intolerance by some patients.Lack of mortality and the absence of obvious signs of acute intoxication at doses up to 5000 mg/kg suggest that for all practical purposes, the extract of *P. vulgaris* is safe when administered orally (Lorke, 1983).

Triton WR-1339 (nonionic detergent) is a known agent for the induction of acute hyperlipidemia due to its reported role in inhibiting the activity of the enzyme lipoprotein lipase (Abdou, 2018). It also interferes in the uptake of triacylglycerol-rich lipoprotein from plasma by peripheral tissues (Zarzecki *et al.,* 2014). Consistent with this, there were marked elevation in total cholesterol, triglyceride, LDL-C and VLDL-C with decreased levels of DHL-C in rats treated with Triton WR-1339. These biomarkers have been shown to be associated with cardiovascular diseases (Upadhyay, 2015). The ability of the extract and its fractions to lower cardiovascular risk lipid biomarkers with elevation of cardio-protective lipid (HDL-C) in Triton WR-1339 induced hyperlipidemia shows that *P. vulgaris* may be beneficial in the prevention and management of risk factors associated with cardiovascular diseases. The lipid lowering effect of *P. vulgaris* may be due to its secondary metabolites like saponins, tannins and flavonoids which have been shown in similar studies to exhibit antihyperlipidemic activities. Saponins are known to facilitate removal of free fatty acid from circulation and to decrease total cholesterol through increase in lipoprotein lipase activity and inhibition of fatty acid synthesis (Khan *et al.,* 2015). Tannins belonging to the flavan-3-ol and gallotannin groups have been found to inhibit cholesterol biosynthesis through suppression of HMG-COA reductase enzyme activity (Chang *et al.,* 2001). Inhibition of cholesterol biosynthesis has also been reported for flavonoids (Niu *et al.,* 2015).

Increase in HDL-C (cardio-protective lipid) is an extra observed beneficial effect of the extract and fractions of *P. vulgaris*. HDL facilitates the translocation of cholesterol from peripheral tissue to liver for catabolism thereby providing a positive effect on lipid metabolism (Cruz *et al.,* 2013). This effect is beneficial in slowing down atherosclerotic process associated with cardiovascular diseases. The increase in HDL-C by *P. vulgaris* may have been mediated through increase in the activity of lecithin cholesterol acyltransferase, which play a key role in incorporating free cholesterol to HDL for hepatic transport. Flavonoids have been shown to enhance the activity of LCAT (Srinivasan and Pari, 2013) and may account for the observed higher HDL by the extracts of *P. vulgaris*.

Triton X-100 belongs to the same group of non-ionic detergent as Triton WR-1339 and as such show similar pattern of induction of hyperlipidemia. Poor eating habits involving high dietary fats have been linked to increased incidence of cardiovascular diseases and metabolic disorders (Lawrence, 2013). High fat diet induced experimental hyperlipidemia therefore mimics natural human dietary cause of abnormal lipid metabolism. The decreased cholesterol and triglyceride levels along with LDL-C and VLDL-C which were evidence from the result of the chronic hyperlipidemic study is a sign that *P. vulgaris* may mediate in lipid disorders associated with hyperlipidemic diet. These effects could be due to increased cholesterol excretion and/or decreased cholesterol absorption through inhibition of pancreatic lipase enzyme activity. Saponins and flavonoids have both been demonstrated to ameliorate high fat diet induced hyperlipidemia through these above-mentioned mechanisms (Lawrence, 2013, Lunagariya *et al.,* 2014). These phytocompound in *P. vulgaris* may account for its lipid lowering effect. Enhanced transport of lipids is a known essential role of HDL (Cruz *et al.,* 2013). The elevation of this lipoprotein in *P. vulgaris* treated hyperlipidemic rats may have contributed to the overall observed hypolipidemic activity of the extracts.

Catechin, detected through HPLC fingerprinting as the most abundant compound in the extract of *P. vulgaris* is a well-documented phytocompound that interferes in abnormalities of lipid metabolism associated with hyperlipidemia (Ahmad *et al.,* 2015). The processes of lipid absorption, transport and excretion are all modulated by catechin (Velayutham *et al.,* 2008). Some catechins like epigallocatechin-3-gallate (EGCG) are poorly absorbed orally (Smith, 2011). Their high concentrations in the intestinal lumen favour their formation of insoluble co-precipitates of lipids like cholesterol thus hindering their absorption. They also interfere with specific transport proteins on the brush border membranes that play significant role in the uptake of lipids by enterocytes (Alkhafaji and Latif, 2012). Furthermore, catechins up-regulate hepatic LDL-receptor expression and its binding activities thereby modulating the excretion and intracellular processing of lipids (Stangl *et al.,* 2006). Considering the multiple hypolipidemic

targets of catechins, they may therefore be hypothesized at least in part to be responsible for the antilipidemic effect of *P. vulgaris*.

The important role played by oxidative stress in vascular damage and progression of various vascular diseases including atherosclerosis, hypertension and congestive heart failure has been demonstrated through many experimental evidences (Lakshmi *et al.,* 2009). The resulting discoloration of the deep purple solution of DPPH is stoichiometric with respect to the number of electrons taken up by the DPPH radical. The number and arrangements of phenolic hydroxyl groups make phenolic compounds like catechin an excellent electron donors and thus efficient scavengers of free radicals (Lee *et al.,* 2014). The abundance of this compound in *P. vulgaris* might have influenced its antioxidant activity which may play a beneficial role in oxidative complications associated with cardiovascular disorders.

Increased vascular oxidative stress in hyperlipidemia is associated with endothelial dysfunction and atherogenesis (Stapleton *et al.,* 2010). High availability of oxidizable lipids substrates like LDL in hyperlipidemic state, makes lipid peroxidation an important oxidative marker of vascular disorders associated with cardiovascular diseases (Gradinaru *et al.,* 2015). Further evidence that lipid peroxidation is facilitated in hyperlipidemia is provided by studies showing positive correlation between cholesterol concentration and the content of cholesteryl derived peroxides (Moriel *et al.,* 2000). The extracts of *P. vulgaris* through it observed inhibition of lipid peroxidation may be beneficial in providing vascular defence against oxidative stress. Inhibition of lipid peroxidation by *P. vulgaris* may also contribute to reduction of hyperlipidemic induced LDL oxidation in the vasculature thereby limiting or preventing the progression of atherosclerosis.

Initiation and progression of oxidative stress are usually associated with reduced endogenous antioxidant enzymes. Several studies have found that catechins up-regulate antioxidant enzymes including glutathione peroxidase and catalase (Yonekura *et al.,* 2016). Glutathione peroxidase is an important enzyme in cellular antioxidant defence system that contributes to detoxification of peroxides and hydroperoxides including those formed as products of lipid peroxidation. The up-regulation of this enzyme by *P. vulgaris* may have contributed to the inhibition of lipid peroxidation recorded in this study. Other endogenous antioxidant enzyme like catalase is important in decomposition of highly reactive hydrogen peroxide radicals. Oxidative stress by hydrogen peroxide is associated with increased phosphorylation of tyrosine kinases which may lead to stronger binding of neutrophils cells on endothelium leading to endothelial dysfunction that predispose to atherogenesis (Bourcier *et al.,* 1997). Production of transcription factors such as nuclear factor Kappa B (NF-kB) and activator protein-1 which participate in the expression of adhesion molecules like vascular cellular adhesion molecule (VCAM-1), intracellular adhesion molecules (ICAM-1), E-selectin and other cytokines are added mechanisms through which hydrogen peroxide mediates atherogenesis (Vogiatzi *et al.,* 2009). Induction of catalase enzyme may therefore intervene in many processes leading to atherogenesis and may contribute to the role of *P. vulgaris* use in the management of cardiovascular disorders.

Catechin, the abundant compound detected in the extract of *P vulgaris* have also been shown by other studies to exhibit other antioxidant properties like chelation of redox active transition-metal ions, inhibition of redox sensitive transcription factors and inhibition of pro-oxidant enzymes (Venkateswara *et al.,* 2011). The antioxidant effects of catechin have been demonstrated to play a major role in mediating the cardioprotective role of natural products like green tea (Velayuthan *et al.,* 2008). It is hypothesized that the abundant of this compound in *P. vulgaris* may have contributed to its antioxidant and antihyperlipidaemic activities and thus its ethnomedicinal application in the management of cardiovascular disorders.

CONCLUSION

The extract of *Phaseolus vulgaris* showed both anti-hyperlipidemic and antioxidant activities with the ethylacetate fraction showing comparably higher effects among all solvent fractions. These activities may be connected to the abundance of catechin phytocompound in the extract as detected through HPLC fingerprinting. The antihyperlipidemic and antioxidant activities may explain the use of this plant in the alternative management of cardiovascular disorders in folklore medicine. Hence, *Phaseolus vulgaris* can be exploited as an alternative antihyperlipidemic and antioxidant agent or as an adjunct to existing therapy in the treatment of hyperlipidemia.

CONFLICT OF INTEREST

The authors in conducting this research and writing the report declare that there were no conflicting interests.

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