Nigerian Journal of Biochemistry and Molecular Biology

The Official Publication of the Nigerian Society of Biochemistry & Molecular Biology (NSBMB). Journal homepage: https://www.nsbmb.org.ng/journals

e-ISSN: 2659-0042

Research Article

In vitro Antioxidant Activities and Blood Protective Effects of Aqueous Extracts of *Xylopia aethiopica* L. Whole Seed and Pod

Habibu Tijjani[1,2*], Aliyu A. Omar[2], Abubakar Mohammed[3], Fatima M. Habibu[1,2], Yohanna B. Maina[1,2], Ali Musa[2]

[1]*Department of Biochemistry, Natural Product Research Laboratory, Bauchi State University, Gadau, Bauchi State, Nigeria*

[2]*Department of Science Laboratory Technology, Bauchi State University, Gadau, Bauchi State, Nigeria*

[3]*Department of Biochemistry, Federal University Lafia, Lafia, Nasarawa State, Nigeria*

OPEN ACCESS

***CORRESPONDENCE**
Tijjani, H.
habibtijjani@basug.edu.ng

ARTICLE HISTORY
Received: 12/11/2021
Reviewed: 25/01/2022
Revised: 07/02/2022
Accepted: 20/02/2022
Published: 02/03/2022

CITATION
Tijjani, H., Omar, A.A., Mohammed, A., Habibu, F.M., Maina, Y.B and Musa, A. (2022). *In vitro* antioxidant activities and blood protective effects of aqueous extracts of *Xylopia aethiopica* L. whole seed and pod. *Nigerian Journal of Biochemistry and Molecular Biology*. 37(1), 48-57

ABSTRACT

Xylopia aethiopica L. (*Annonaceae*) is medicinally important in the treatment of a wide range of diseases. In Nigeria, *X. aethiopica* whole seed are locally used in the treatment of constipation. They are used as a tonic tea and beverages, and after pregnancy delivery to facilitate the removal of clotting blood in the system. The study evaluated *in vitro* antioxidant activities and blood protective effects of *X. aethiopica* aqueous extracts. The 2,2-diphenyl-1-picrylhydrazyl scavenging activities of the seed (IC_{50} = 0.19±0.03 mg/mL) and pod (IC_{50} = 0.24±0.02 mg/mL) extract were not significantly ($p>0.05$) different with butylated hydroxytoluene BHT (IC_{50} = 0.17±0.04 mg/mL) and ascorbic acid (IC_{50} = 0.13±0.05 mg/mL). Furthermore, total antioxidant capacity and nitric oxide reducing power of the seed, pod and whole seed were similar with no significant difference ($p>0.05$) when compared with BHT. However, the seed (IC_{50} = 0.89±0.09 mg/mL), pod (IC_{50} = 0.35±0.03 mg/mL) and whole seed (IC_{50} = 0.32±0.03 mg/mL) expressed significantly ($p<0.05$) lower hydrogen peroxide decomposing activity when compared with BHT (IC_{50} = 0.15±0.00 mg/mL) and ascorbic acid (IC_{50} = 0.15±0.01 mg/mL). The extract expresses no erythrocyte lysis activities at a concentration range of 6.25 – 100 µg/mL and protect against accumulation of $CuSO_4$-induced conjugated dienes in plasma. The present study demonstrated the protective nature of *X. aethiopica* L. seed, pod and whole seed extracts against the accumulation of conjugate dienes, thrombolytic activities and membrane stability effects. *X. aethiopica* L. is recommended for consideration as a significant natural antioxidant source.

Keywords: *Xylopia aethiopica, Antioxidant activities, Plasma; Oxidation, Whole Seed, Pod, GCMS analysis*

INTRODUCTION

The blood comprises of several components which includes the red blood cells (RBCs), white blood cells (WBCs) and in clinical investigations could be prepared into plasma or serum, depending on the required investigations. The RBCs (erythrocytes) are responsible for the delivery of oxygen received from the lungs to body tissues. The shape and structure of the RBCs are required for their carrier properties; their deformability affects whole blood viscosity, particularly at microcirculation where the capillary diameter is smaller than the erythrocytes diameter (Bosman *et al.*, 1995; Girasole *et al.*, 2007). The blood cell membrane contains lipids, which serve as a lipid bilayer preventing the unwanted movements of proteins, ions and other molecules outside the blood cell. The lipids are rich in saturated or unsaturated fatty acids, and are susceptible to oxidation by free radicals (Nabavi *et al.*, 2009). Previous studies have focused on the effects of free radicals associated peroxidation of membrane lipids leading to a variety of pathological diseases (Dröge, 2002; Valko *et al.*, 2007; Pham-Huy *et al.*, 2008). Natural and synthetic antioxidants have been used to prevent the deleterious effects of free radicals on membrane lipids and avoid free radical chain reactions (Adom and Liu, 2005). Traditionally, plants and their extract are used to treat blood related disorders. Such plant includes *Xylopia aethiopica* L. (*Annonaceae*) which is

used as a tonic tea and beverages, and after pregnancy delivery to facilitate the removal of clotting blood in the system.

X. aethiopica is medicinally important in the treatment of a wide range of diseases. In Nigeria, the local names include *Eeru, Uda* and *Kimba* in Yoruba, Igbo and Hausa respectively (Orwa *et al.,* 2009). Other common names include African pepper, Senegal pepper, West African pepper, negro pepper and spice tree (Jirovetz *et al.*, 1997). *X. aethiopica* is used traditionally for the treatment of amenorrhea, biliousness, bronchitis, cough, dysentery, malaria, rheumatism and uterine fibroid (Burkill, 1995). The fruits extracts possess antimicrobial activities against gram positive and gram negative bacteria (Tatsadijieu *et al.*, 2004).

Adverse effects of free radicals and reactive oxygen species (ROS) are continuously generated in human system and their mechanisms of generations and multiplication have been implicated in several pathological conditions of several human diseases (Aruoma, 1998). Antioxidants are beneficial in such disease conditions as they decrease cell degeneration and death through the chelating of free radicals (Gülçin, 2012). More so, the beneficial effects of antioxidants are through the prevention of free radicals from reacting with cell membranes and their components, such as fatty acids, lipids, proteins, carbohydrates, and nucleic acids (Gülçin, 2012). *X. aethiopica* contains appreciable essential oils with reported antioxidant activities (Koroch *et al*., 2007). The essential oils are similarly antimicrobial, and antiviral (Koroch *et al.,* 2007). The reported antioxidant activities of essential oils of *X. aethiopica* are associated α and β pinenes, 1,8 cineole and sabinene as reported in the fresh, dried fruits, and essential oil extracts from the leaves and fruits of *X. aethiopica* (Karioti *et al.,* 2004; Odukoya *et al.,* 2008). Furthermore, other beneficial effects of *X. aethiopica* whole seed include their use in the treatment of constipation, different stages of syphilis, rheumatism, and inflammatory conditions (Tatsadijieu *et al.,* 2004). This study aims at evaluating the GCMS phytochemical constituents, *in vitro* antioxidant activities and blood protective effects of *X. aethiopica* seed, pod and whole seed aqueous extracts following their reported traditional usage.

MATERIALS AND METHODS

Chemicals and Reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), $CuSO_4$, hydrogen peroxide (H_2O_2), phosphomolybdenum, vitamin C, triton X-100 and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Company, St. Louis, MO, USA. Acetyl salicyclic acid and streptokinase were purchased from Swiss Pharma, Gujarat, India. All other chemicals were of analytical grades.

Plant Material

Xylopia aethiopica whole seed was purchased from Azare market, Bauchi State, Nigeria and identified at the Department of Biological Sciences, Bauchi State University, Gadau. A voucher sample was deposited at the herbarium with the Federal College of Forestry, Jos, Plateau State.

Preparation of Plant Material

Xylopia aethiopica whole seed were divided into 3 portions. The seed was separated in the first portion, the pods in the second portion, while the whole seed was used in the third portion. The aqueous extract of *X. aethiopica* was prepared by dissolving fifty grams (50 g) each of the powdered portions separately in 250 mL distilled water for 24 hours in a tightly stopped glass container. After which the samples were filtered using Whathman filter paper No. 1. The filtrates were concentrated separately using a rotary evaporator at 40^{o}C, and generated the crude extracts used for the study.

Animal Care

Eight (8) albino mice of both sexes were purchased from the Pharmacology Animal Unit, Bauchi State University. They were also allowed free access to standard mice feed and water *ad libitum*. The animals were sacrificed in batches under diethyl ether anesthesia to prepare serum, plasma and blood cells. The University of Jos Ethical Review Committee approved the research procedures with the registration number UJ/FPS/F17-00379.

GCMS Analysis

The identification of phytochemical constituents was carried out using Gas Chromatography Mass Spectrometry (GC-MS), Agilent-7890A (GC) instrument coupled with a Mass Spectrometer detector. The results were presented as percentage identified components, based on the comparison of retention indices, component names, peak number, retention time, peak height and phytochemical area.

In vitro Antioxidant Assay

The *in vitro* antioxidant scavenging activity against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical was determined according to the method described by Turkoglu *et al*. (2007), while total antioxidant capacity (TAC) was determined using the phosphomolybdenum method as described by Prieto *et al*. (1999). Nitric oxide (NO) scavenging capacity was determined according to the method of Fiorentino *et al*. (2008) while hydrogen peroxide (H_2O_2) scavenging activity was determined according to the method described by Ruch *et al*. (1989).

Erythrocyte Lysis Assay

The erythrocyte lysis of the various extracts were performed using 100 µL of Packed RBC (~10^9 cells/ml) prepared from mice. The test tubes conditioning RBC were incubated with 90 µL PBS (pH 7), 10 µL of the extract while the negative control contains 10 µL of 1% Sodium dodecyl sulphate (SDS). After incubation for 15 minutes at 37ºC, the tubes were centrifuged for 10 minutes at 13000 rpm. The supernatant obtained was then diluted 1/200 with PBS in triplicates. The absorbance was read against the PBS blank at 410 nm.

Conjugate Dienes Determination Assay

Concentrations of conjugate dienes were determined in plasma samples using the method described by Liao *et al*. (2014). Plasma samples were diluted 40 folds using PBS to obtain 2.5% plasma concentration, followed by treatment with 1 mL of PBS, vitamin C, BHT and *X. aethiopica* extracts at 6.25 - 100 µg/mL and incubated for 20 munities at 37ºC with gentle shaking. 2 mL of 200 µM $CuSO_4$ was added to each test tube and incubated for another 20 minutes at 37ºC with gentle shaking. Conjugate Dienes were determined after every 30 minutes by measuring absorbance at 245 nm.

Erythrocytes Protection Assay

Erythrocytes protection through the inhibition of erythrocyte haemolysis was assayed based on the method of Cheung *et al*. (2003) as modified by Liao *et al*. (2014). Plasma from experimental animals was washed three times using PBS (pH 7.4) and diluted to 20% erythrocyte suspension. 0.2 mL of suspension was incubated with 0.2 mL various *X. aethiopica* extract at 37°C for 20 minutes with gentle shaking, after which 0.4 mL of 2,2- Azobis(2-amidinopropane) Dihydrochloride (AAPH, 200 mM) was added, and the incubation continued at the same temperature for 2 hours. The reaction mixture was then diluted with 8 mL of PBS (8 mL of ultrapure water for negative control) and centrifuged for 10 minutes at 1200 xg. The absorbance of the supernatant was measured at 540 nm.

Thrombolytic Activity Assay

The method described by Prasad *et al*. (2007) was used to evaluate the thrombolytic activities of *X. aethiopica* extract. About 1 mL of experimental animal blood were pre-weighed and allowed to clot at 37°C for about 45 minutes. The serum was removed and test tubes were weighed again to determine clot weight. 100 µL of distilled water, streptokinase (equivalent to 30,000 IU) and various *X. aethiopica* extract (2 mg/mL) were added to the test tubes for negative non-thrombolytic control, standard and test respectively. The mixtures were incubated for 90 minutes at 37°C. The released fluids from the clot were removed and the test tubes weighted again. The thrombolytic activities were then calculated.

Membrane Stability Assay

Membrane stability effects of various extracts of *X. aethiopica* extract were determined according to the method described by Shinde *et al.* (1999) and Sikder *et al*. (2012). Erythrocyte suspension of red blood cells (RBCs) was prepared from EDTA treated blood of experimental mice and successively separated using an isotonic solution of NaCl (154 mM, 10 mM Na_3PO_4, pH 7.4). 0.5 mL of the suspension was mixed with 4.5 mL hypotonic solution (50 mM NaCl, Na_3PO_4 buffer saline, pH 7.4) of *X. aethiopica* extracts (2 mg/mL). The mixture was incubated at room temperature for 10 minutes and centrifuged at 3000 xg for 10 minutes to remove the supernatant. The absorbance of the supernatant was measured at 540 nm.

Statistical analysis

Data were subjected to statistical analysis using one-way analysis of variance followed by Duncan multiple range test (SPSS version 20, SPSS Inc., Chicago. IL, USA). Data (where applicable) are presented as mean ± SEM. Significance levels were considered at $p < 0.05$ while the graphs were generated using GraphPad Prism 6 software (GraphPad Software, California, USA).

RESULTS AND DISCUSSION

The aqueous whole seed extract of *X. aethiopica* contains several bioactive components (Figure 1). About 501 peaks were recorded; top 16 phytochemicals with appreciable percentage concentrations are presented in Table 1, while Figure 2 presents the structures of some identified phytochemicals. 1,4-dioxaspiro[2.4]heptan-5-one, 2h-pyran-2,6(3h)-dione, 7,7-dimethyl- 3,4-dimethyldihydrofuran-2,5-dione, 7,7-dimethyl- 3-buten-1-ol and distannoxane were found in high concentration in the aqueous whole seed extract of *X. aethiopica*. Ethnobotanically, plants and their extracts are used for treatment and management of diseases since ancient times (Woode *et al.*, 2011). About 80% of the health needs of the rural populace in most regions of Africa are met through traditional medicine. *X. aethiopica* is one of the medicinal plants, whose parts are of high medicinal value in many countries of Africa (Eze, 2012). These plants are widely accepted due to the valuable secondary metabolites that are contained in them which are used to maintain human health and possess remarkable therapeutic potentials (Kumar *et al.*, 2005; Tijjani *et al.*, 2018). Whole seed extract of *X. aethiopica* contains important secondary metabolites including 1,4-dioxaspiro[2.4]heptan-5-one, 2h-pyran-2,6(3h)-dione, 7,7-dimethyl- 3,4-

dimethyldihydrofuran-2,5-dione, 7,7-dimethyl- 3-buten-1-ol and distannoxane, which were found in high concentration. The rich phytochemical contained in *X. aethiopica* could be responsible for not only the biochemical activities of the plant but also the taste and aroma associated with the plant.

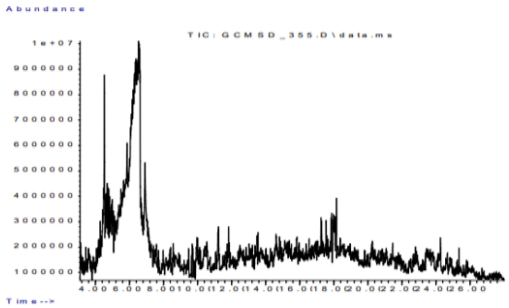


Figure 1. GCMS Chromatogram of Aqueous Whole Seed Extract of *Xylopia aethiopica*

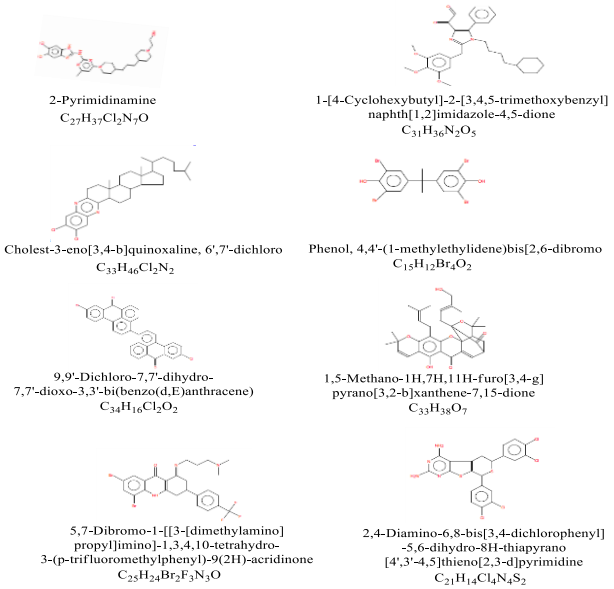


Figure 2. Chemical Structures of Some Identified Phytochemicals Through GCMS Analysis of Aqueous Whole Seed Extract of *Xylopia aethiopica*

The extracts from *X. aethiopica* whole seed, pod and seed expressed *in vitro* antioxidant activities (Figure 3-6, Table 2). In DPPH assay, aqueous extract of *X. aethiopica* seed and pod expressed IC_{50} values of 0.19±0.03, and 0.24±0.02 mg/mL, with no significant difference ($p>0.05$) compared with BHT and vitamin C with IC_{50} values of 0.17±0.04 and 0.13±0.05 mg/mL respectively (Figure 3, Table 2). No significant difference ($p>0.05$) was observed in the TAC and NO activities of the extracts and BHT. However, the IC_{50} values of the extracts for H_2O_2 assay are significantly higher ($p<0.05$) compared with BHT and vitamin C. The effects of oxidation are among the leading causes of progression in some diseases (Pham-Huy *et al.*, 2008).

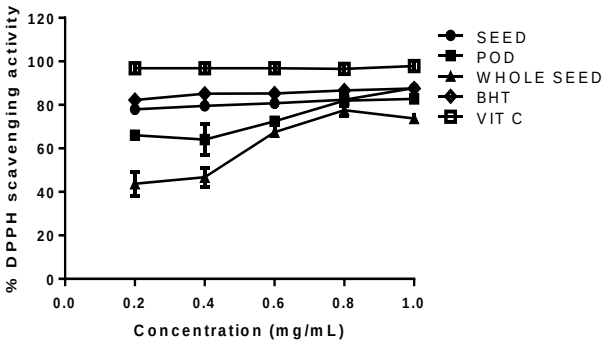


Figure 3. DPPH Scavenging Activities of Aqueous Extracts of *Xylopia aethiopica*. Values are means ± SEM of triplicate determinations BHT = Butylated hydroxytoluene, Vit C = Ascorbic acid

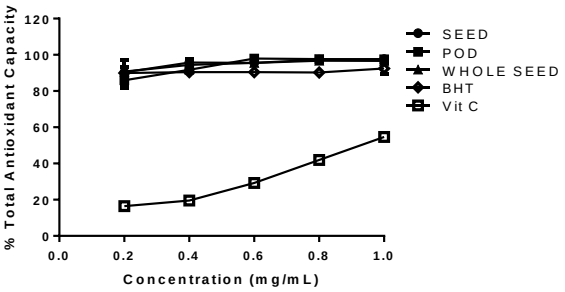


Figure 4. Total Antioxidant Capacity of Aqueous Extracts of *Xylopia aethiopica*. Values are means ± SEM of triplicate determinations. BHT = Butylated hydroxytoluene, Vit C = Ascorbic acid.

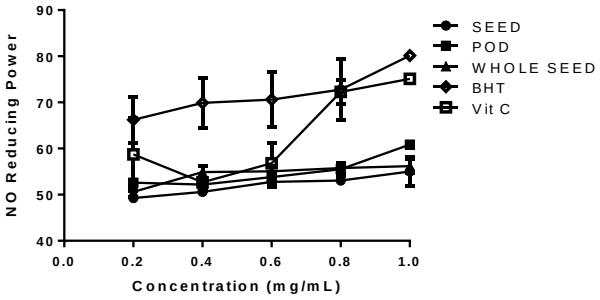


Figure 5. Nitric Oxide Reducing Power of Aqueous Extracts of *Xylopia aethiopica*. Values are means ± SEM of triplicate determinations. BHT = Butylated hydroxytoluene, Vit C = Ascorbic acid.

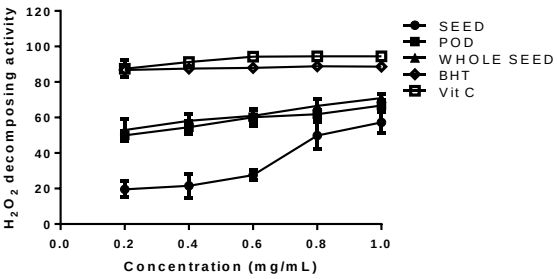


Figure 6. Hydrogen Peroxide Decomposing Activity of Aqueous Extracts of *Xylopia aethiopica*. Values are means ± SEM of triplicate determinations. BHT = Butylated hydroxytoluene, Vit C = Ascorbic acid.

Table1. Abundant Phytochemical Constituents of Aqueous Whole See Extract of *Xylopia aethiopica*

S/No	Components	Peak	Retention Time	Peak Height	Area	Concentration (%)
1.	1,4-Dioxaspiro[2.4]heptan-5-one	54	5.660	3887371	238155285	4.412%
2.	2H-Pyran-2,6(3H)-dione	83	6.918	4487214	301977223	1.908%
3.	7,7-dimethyl- 3,4-Dimethyldihydrofuran-2,5-dione	58	5.866	5349087	205406354	1.298%
4.	7,7-dimethyl- 3-Buten-1-ol	55	5.751	3740069	164578485	1.040%
5.	Distannoxane	32	4.522	7775866	159485775	1.008%
6.	Hydroxy-1-Hexene	56	5.799	3951272	114781111	0.725%
7.	1-[p-Tolylsulfonyl]-2-methylaziridine	46	5.185	2285414	101597383	0.642%
8.	Cyclobutane	84	7.017	2388672	87723056	0.554%
9.	2-Pyridinemethanamine	247	15.124	1514410	83837217	0.530%
10.	Propanedioic acid	315	18.238	1310049	77986841	0.493%
11.	2-Azetidinone	85	7.100	2028410	69044415	0.436%
12.	Z-28-Heptatriaconten-2-one	298	17.522	1365746	66147289	0.418%
13.	2,2-Dibromocholestanone	218	13.714	1230366	64236726	0.406%
14.	3-Amino-2,2-dimethyl-1-propanol	50	5.373	2861790	63819593	0.403%
15.	Cyanoacetic acid	91	7.296	1352087	63830626	0.403%
16.	Oleanan-29-oic acid	35	4.646	3290260	57646310	0.364%

Table 2. IC_{50} Values for Various *In vitro* Antioxidant Activity of Aqueous Extracts of *Xylopia aethiopica*

	IC_{50} (mg/mL)			
Samples	**DPPH**	**TAC**	**NO**	**H_2O_2**
Seed	0.19 ± 0.03^{ab}	0.14 ± 0.01^{a}	0.39 ± 0.01^{a}	0.89 ± 0.09^{a}
Pod	0.24 ± 0.02^{a}	0.14 ± 0.00^{a}	0.37 ± 0.01^{a}	0.35 ± 0.03^{b}
Whole Seed	0.35 ± 0.01^{c}	0.15 ± 0.01^{a}	0.35 ± 0.01^{a}	0.32 ± 0.03^{b}
Butylated hydroxytoluene	0.17 ± 0.04^{b}	0.15 ± 0.00^{a}	0.30 ± 0.05^{a}	0.15 ± 0.00^{c}
Ascorbic acid	0.13 ± 0.05^{ab}	0.94 ± 0.00^{b}	0.34 ± 0.02^{a}	0.15 ± 0.01^{c}

Values are means ± SEM of triplicate determinations. **DPPH** = 2,2-diphenyl-1-picrylhydrazyl scavenging activities, **TAC** = Total antioxidant capacity, **NO** = Nitric oxide reducing power, **H_2O_2**= Hydro peroxide decomposing activity, **IC_{50}**= Half maximal inhibitory concentration.

Antioxidants prevent the cellular damages and disease progressions that could arise by oxidation from free radicals. *X. Aethiopica* possesses good *in vitro* antioxidant activities compared with the reference compounds, vitamin C and BHT. Among the effective antioxidant activities of the extract is nitric oxide and 2,2-Diphenyl-1-picrylhydrazyl scavenging activities. The hydrogen per oxide scavenging of the whole seed and pod of *X. aethiopica* were significantly ($p<0.05$) higher compared with the seed extract. Hydroxyl radical is the most reactive of the reactive oxygen species (ROS), with a short half-life compared with the other ROS (Amudha and Rani, 2016). Hydroxyl radical which could be generated from hydrogen peroxide are capable of oxidative damages to lipids, proteins or DNA. *X. aethiopica* ability to cause significant NO and DPPH scavenging activities could be their ability to donate hydrogen (Chebouat *et al.,* 2011), thereby protecting membrane lipids and proteins, and prevention of DNA damage (Olanlokun *et al.,* 2020).

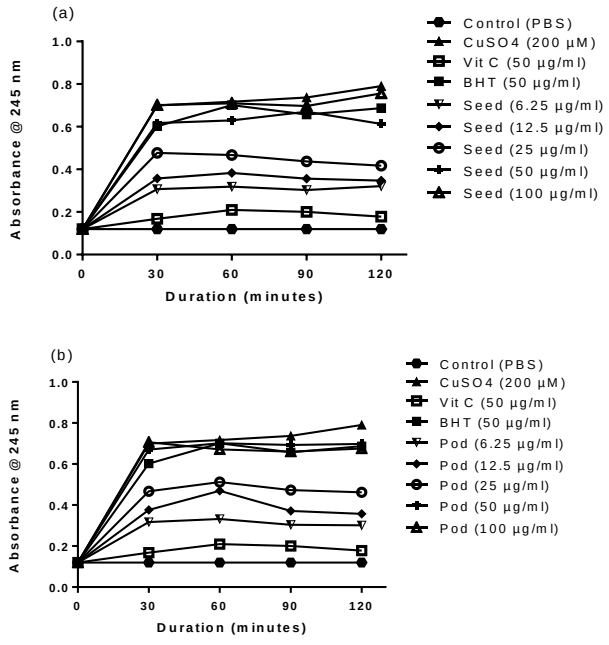
The integrity of erythrocyte membrane is key to their biological and physiological functions. The erythrocyte membranes contain abundant polyunsaturated fatty acids, which are susceptible to oxidation by free radicals (Nabavi *et al.,* 2009). The ability of RBC to resist osmotic lysis, increases its stability, and prevents against many damaging effects of chemotherapeutic drugs and other unfavourable conditions, including excessive temperature (Paraiso *et al.,*

2017). Aqueous extracts of *X. aethiopica* whole seed, pod and seed protected the RBCs from lysis at concentrations of 6.25 - 100 µg/mL. Haemoglobin (Hb), the main component of the RBC, is primarily responsible for the transportation of oxygen (Ahrens *et al.,* 1993). During RBC hemolysis, Hb is released extracellularly, exposing them for oxidation and preventing them from their function of oxygen transportation. Aqueous extracts of *X. aethiopica* could prevent Hb exposure to oxidation, by preventing RBCs lysis. The extracts from *X. aethiopica* whole seed, pod and seed did not lysis RBCs (Table 3), and protected the cells against $CuSO_4$-induced accumulation of conjugated dienes in plasma at various concentrations at 6.25 - 100 µg/mL (Figure 7).

Table 3. Effects of Aqueous Extracts of *Xylopia aethiopica* on Percentage Erythrocyte Haemolysis.

Samples	% Haemolysis
Control (Phosphate Buffer Saline)	0.00
Control (1% Triton X100)	100.00
Seed (6.25 – 100 µg/mL)	0.00
Pod (6.25 – 100 µg/mL)	0.00
Whole Seed (6.25 – 100 µg/mL)	0.00
Butylated hydroxytoluene (100 µg/mL)	0.00
Ascorbic acid (100 µg/mL)	0.00

Values are means of triplicated determinations.



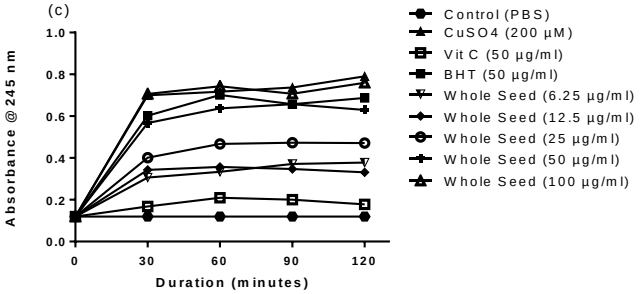


Figure 7. Protective effects of aqueous extracts of Xylopia aethiopica seed (A), pod (B) and seed and pod (C) against CuSO4-induced accumulation of conjugated dienes in plasma. Values are means ± SEM of three determinations. PBS = Phosphate buffer saline, Vit C = Ascorbic acid, BHT = Butylated hydroxytoluene

Furthermore, the extracts of *X. aethiopica* demonstrated good *in vitro* protection of erythrocytes at lower concentrations compared with BHT and vitamin C treatments (Table 4). Experimentally, $CuSO_4$ can be used to generate free radical in plasma from their decomposition at physiological temperature (Zou *et al.,* 2001; Liao *et al.,* 2014), as also used in this study. The formation of conjugate dienes could be measured spectrometrically to access the level of oxidation. *X. aethiopica* seed, pod and whole seed protected against $CuSO_4$-induced accumulation of conjugated dienes in plasma and protected erythrocytes better at lower concentrations compared with BHT and vitamin C treatments. This implies that extracts from *X. aethiopica* can trap peroxyl radical chain and other free radicals, and hence protecting membrane lipids from the deleterious effects of the free radicals (Adom and Liu, 2005). Furthermore, lipoprotein oxidation in plasma is contributing factor of pathological significance of hyperlipidemia and atherosclerosis. Oxidation of low-density lipoprotein cholesterol (LDL-Chol) and high-density lipoprotein cholesterol (HDL-Chol) are contributing factors to the development of atherosclerosis and hypercholesterolemia (Rifici and Khachadurian, 1996).

Thrombolytic assay indicated that *X. aethiopica* whole seed (23.40±04), pod (17.54±05) and seed (20.40±07) expressed significantly lower ($p<0.05$) percentage thrombolysis activities compared with the reference streptokinase (67.05±13) and NaCl (55.15±16) respectively (Table 5). Similarly, membrane stability assay indicated that *X. aethiopica* whole seed (57.83±3.95), pod (54.62±3.27) and seed (58.71±2.38) expressed more potent percentage membrane stability effects, which are significantly lower ($p<0.05$) compared with the reference acetyl salicyclic acid (83.51±4.28) and NaCl (100.00±0.00) respectively (Table 6). Thrombosis is the formation of blood clots, which obstruct the flow of blood in the blood vessels. Thrombosis occurs due to several factors, some of which includes injury to the blood vessels, blood flow interruption in the vessels

and hypercoagulation of blood (Furie and Furie, 2008). It's associated with arthritis, cerebrovascular ischaemia, cancer, myocardial infarction, pulmonary, stroke and venous embolism (Bick and Kaplan, 1998; Furie and Furie, 2008). Drugs such as streptokinase and urokinase are used to treat thrombosis, but are associated with serious bleeding and are expensive treatment methods (Katzung, 2008). *X. aethiopica* seed, pod and whole seed aqueous extracts thrombolysis activities were lower compared with the reference streptokinase.

Table. Erythrocytes Protection Effects of Aqueous Extracts of *Xylopia aethiopica*

Samples (Concentrations)	% Inhibition	Samples (Concentrations)	% Inhibition	Samples (Concentrations)	% Inhibition
Control (PBS)	66.94				
BHT (100)	47.76				
Vit C (100)	46.12				
Seed (6.25)	65.31	Pod (6.25)	76.33	Whole Seed (6.25)	55.92
Seed (12.5)	53.06	Pod (12.5)	48.98	Whole Seed (12.5)	59.18
Seed (25)	71.02	Pod (25)	67.76	Whole Seed (25)	58.78
Seed (50)	64.49	Pod (50)	61.63	Whole Seed (50)	48.98
Seed (100)	30.20	Pod (100)	77.14	Whole Seed (100)	20.41

PBS = Phosphate buffer saline, BHT = Butylated hydroxytoluene, Vit C = Ascorbic acid, Concentrations = µg/mL

Plant extract with thrombolytic properties are associated with their secondary metabolite contents which included alkaloids, flavonoids, tannins and saponins (Rathee *et al.*, 2009; Ali *et al.*, 2013). The aqueous extract of *X. aethiopica* contains several secondary metabolites, which could be responsible for the thrombolytic activities observed in the whole seed, pod and seed extracts. Similarly, the thrombolytic activities of plant extracts and their lipid lowering activities might contribute to healthy cardiovascular system (Doggen *et al.*, 2004).

Membrane stability effects of *X. aethiopica* were significantly higher compared with the reference acetyl salicyclic acid. Membrane stabilization of erythrocytes is an important measure of anti-inflammatory compounds. The disruption of erythrocyte membrane due to treatments with hypotonic or hypertonic solutions results in leakage of serum proteins and fluids into the tissues leading to inflammation (Halliwell and Whiteman, 2004). The results suggest *X. aethiopica* aqueous extract as suitable anti-inflammatory agents (Shinde *et al.*, 1999).

Table 5. Thrombolytic Activity of Aqueous Extracts of *Xylopia aethiopica*

Test Groups	Thrombolysis (%)
H_2O	11.21 ± 04^a
NaCl	55.15 ± 16^b
Streptokinase	67.05 ± 13^b
Seed	20.40 ± 07^c
Pod	17.54 ± 05^c
Whole Seed	23.40 ± 04^c

Values are mean ± SEM of triplicated determinations

Table 6. Membrane Stability Effects of Aqueous Extracts of *Xylopia aethiopica*

Test Groups	Membrane Disruption (%)
NaCl	100.00±0.00[a]
Acetyl salicyclic acid	83.51±4.28[b]
Seed	58.71±2.38[c]
Pod	54.62±3.27[c]
Whole seed	57.83±3.95[c]

Values are mean ± SEM of triplicated determinations

CONCLUSION

The study demonstrated that aqueous extract of *X. aethiopica* contains important secondary metabolites and the plant possesses potent *in vitro* antioxidant properties. The study similarly demonstrated the protective nature of *X. aethiopica* L. seed, pod and whole seed extracts against the accumulation of conjugate dienes, thrombolytic activities and membrane stability. *X. aethiopica* L. is recommended for consideration as a significant natural antioxidant source.

AUTHORS' CONTRIBUTIONS

HT, AAO, AM1 designed the study, FMH, YBM, AM2, HT carried out the laboratory investigations, HT drafted the manuscript, HT, AAO, AM1, FMH, YBM, AM2 proofread and approved the manuscript.

FUNDING STATEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

REFERENCES

Adom, K.K. and Liu, R.H. (2005). Rapid peroxyl radicalscavenging capacity (PSC) assay for assessing both hydrophilic and lipophilic antioxidants. *Journal of Agricultural and Food Chemistry*, 53 (17): 6572–6580.

Ahrens, T., Rutherford, K. and Basham, K.A.R. (1993). Essentials of oxygenation: implication for clinical practice. Jones and Bartlett Publishers.

Ali, M.R., Kuri, S., Das, A. and Islam, M.A. (2013). Preliminary phytochemical screening and in vitro thrombolytic potential of the methanolic extract of *Enhydra fluctuans* Lour (leaves). *International Journal of Pharmamedix India*, 1: 270- 280.

Amudha, M. and Rani, S. (2016). Evaluation of *in vitro* antioxidant potential of *Cordia retusa*. *Indian Journal of Pharmaceutical Science*, 78(1): 80–86.

Aruoma, O. I. (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemists Society*, 75 (2): 199-212.

Bick, R.L. and Kaplan, H. (1998). Syndromes of thrombosis and hypercoagulability: congenital and acquired causes of thrombosis. *Medical Clinics of North America*, 82: 409–458.

Bosman, J., Tangelder, G.J., Egbrink, M.G., Reneman, R.S. and Slaaf, D.W. (1995). Red blood cell flow cessation and diameter reductions in skeletal muscle capillaries *in vivo* - the role of oxygen. *Pflugers Archiv : European Journal of Physiology*, *430*(5): 852–861.

Burkill, H. (1995). The useful plants of west tropical Africa. Families JL. Kew. Royal Gardens kew xi ISBN 1995;3:857.

Chebouat, E., Dadamoussa, B., Gharabli, S., Gherraf, N., Allaoui, M., Cheriti, A., Lahham, A. and and Zellagui, A. (2011). Assessment of antimicrobial activity of flavonoids extract from *Ephedra alata*. *Der Pharmacia Lettre*, 6(3):27-30.

Cheung, L.M., Cheung, P.C. andOoi, V.E. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry*, 81: 249−255.

Doggen, C.J., Smith, N.L., Lemaitre, R.N., Heckbert, S.R., Rosendaal, F.R. and Psaty, B.M. (2004). Serum lipid levels and the risk of venous thrombosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 24:1970–5.

Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Reviews*, *82*(1): 47–95.

Eze, K.N. (2012). Antifertility effects of ethanolic extract of *Xylopia aethiopica* on male reproductive organ of wistar rats. *American Journal of Medicine and Medical Sciences*, 2(1): 12-15.

Fiorentino, A., Ricci, A., D'Abrosca, B., Pacifico, S., Golino, A., Letizia, M., *et al*. (2008). Potential food additives from Carexdistachya roots: Identification and in vitro antioxidant properties. J Agric Food Chem., 56:8218-25.

Furie, B. and Furie, B.C. (2008). Mechanisms of thrombus formation. *The New England Journal of Medicine*, 359: 938–949.

Girasole, M., Pompeo, G., Cricenti, A., Congiu-Castellano, A., Andreola, F., Serafino, A., Frazer, B.H., Boumis, G. and Amiconi, G. (2007). Roughness of the plasma membrane as an independent morphological parameter to

study RBCs: a quantitative atomic force microscopy investigation. *Biochimica et Biophysica Acta*, 1768: 1268-1276.

Gülçin, I. (2012). Antioxidant Activity of Food Constituents: An Overview. *Archives of Toxicology*, 86 (3): 345–91.

Halliwell, B, and Whiteman, M. (2004). Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? *British Journal of Pharmacology*, 142:231–55.

Jirovetz, L., Buchbauer, G. andNgassoum, M. (1997). Investigation of the essential oils from the dried fruits of *Xylopia aethiopica* (West African "Peppertree") and *Xylopia parviflora* from Cameroun. *Ernährung/Nutrition*, 21: 324-325.

Karioti, A., Hadjipavlou-Litina, D., Mensah, M.L., Fleischer, T.C. and Skaltsa, H. (2004). Composition and antioxidant activity of the essential oils of *Xylopia aethiopica* (Dun) A. Rich. (*Annonaceae*) leaves, stem bark, root bark, and fresh and dried fruits, growing in Ghana. *Journal of Agricultural and Food Chemistry*, 29;52(26):8094-8.

Katzung, B.G. (2008). Basic and clinical pharmacology. McGraw-Hill Education (Asia), 10th Edition. P. 545.

Koroch, A.R., Juliani, H.R. and Zygadlo, J.A. (2007). Bioactivity of essential oils and their components. In: Berger R.G (ed) flavours and fragrances. Chemistry, bioprocessing and sustainability. Springer-Verlag Berlin Heidelberg.

Kumar, R.S., Sivakuma, T., Sunderem, R.S., Gupta, M., Murugesh, K., Rajeshwa, Y., Kumar, M.S. and Kumar, K.A. (2005). Antioxidant and antimicrobial activities of *Bauhinia recemosa*L. stem bark. *Brazilian Journal of Medical and Biological Research*, 38: 1015 1024.

Liao, W., Ning, Z., Chen, L., Wei, Q., Yuan, E., Yang, J. and Ren, J. (2014). Intracellular antioxidant detoxifying effects of Diosmetin on 2,2- azobis(2-amidinopropane) dihydrochloride (AAPH)-induced oxidative stress through inhibition of reactive oxygen species generation. *Journal of Agricultural and Food Chemistry*, 62: 8648−8654.

Nabavi, S.M., Ebrahimzadeh, M.A., Nabavi, S.F., Fazelian, M. and Eslami, B. (2009). *In vitro* antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. *Pharmacognosy Magazine*, 5:122-6.

Odukoya, O.A., Inya-Agba, S.I., Segun, F.I., Sofidiya, M.O. and Ilori, O.O. (2007). Antioxidant activity of selected Nigerian green leafy vegetables. *American Journal of Food Technology*, 2:169-175.

Olanlokun, J.O., Lawal, O., Babarinde, C.O. and Olorunsogo, O.O.(2020). erythrocyte membrane stabilisation, protease activities and antioxidant properties of the stem bark extract of *Alstoniaboonei* (DC). *Archive of Basic and Applied Medicine*, 8:35 – 44.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Simons, A. (2009). Agroforestree database a tree reference and selection guide version 4.0.

Paraiso, L.F., Gonçalves, E., Oliveira, A.F.M., Cunha, L.M., De Almeida Neto, O.P., Pacheco, A.G., Araújo, K.B.G., GarroteFilho, M.S., Neto, M.B. and Penha-Silva, N. (2017). Effects of acute and chronic exercise on the osmotic stability of erythrocyte membrane of competitive swimmers. *PLoS ONE*, 12: e0171318.

Pham-Huy, L.A., He, H. and Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science : IJBS*, *4*(2), 89–96.

Prasad, S., Kashyap, R.S., Deopujari, J.Y., Purohit, H.J., Taori, G.M. andDaginawala, H.F. (2007). Effect of *Fagonia Arabica* (Dhamasa) on in vitro thrombolysis. *BMC Complementary and Alternative Medicine*, 7:36.

Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, 269: 337-41.

Rathee, P., Chaudhary, H., Rathee, S., Rathee, D., Kumar, V. and Kohli, K. (2009). Mechanism of action of flavonoids as anti-inflammatory agents: a review. *Inflammation and Allergy - Drug Targets*, 8:229–35.

Rifici, V.A. and Khachadurian, A.K. (1996). Effects of dietary vitamin C and E supplementation on the copper mediated oxidation of HDL and on HDL mediated cholesterol efflux. *Atherosclerosis*, 127: 19−26.

Ruch, R.J., Cheng, S.J. and Klaunig, J.E.(1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 10: 1003–1008.

Shinde, U.A., Phadke, A.S., Nair, A.M., Mungantiwar, A.A., Dikshit, V.J. and Saraf, M.N. (1999). Membrane stabilizing activity — a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia*, 70:251–7.

Sikder, M., Millat, M. and Sultana, A. (2012). *In vitro* membrane stabilizing activity total phenolic content, cytotoxic, thrombolytic and antimicrobial activities of *Calliandrasurinamensis* (Wall.). *Journal of Pharmacognosy and Phytochemistry*, 1:40–44.

Tatsadjieu, L. N., Essia Ngang, J. J., Ngassoum, M. B. and Etoa, F. X. (2003). Antibacterial and antifungal activity of Xylopia aethiopica, *Monodora myristica*, *Zanthoxylum xanthoxyloides* and *Zanthoxylum leprieurii* from Cameroon. Fitoterapia, 74(5), 469–472.

Tijjani, H., Egbuna, C. and Luka, C.D. (2018). Biosynthesis of phytochemicals, in phytochemistry, volume 1, chapter

2, fundamentals, modern techniques, and applications, Apple Academic Press Inc. Canada. Pp 37-78.

Turkoglu, A., Duru, M.E., Mercan, N., Kivrak, I. and Gezer, K. (2007). Antioxidant and antimicroial activities of *Laetiporus sulphureus* (Bull.) Murrill. *Food Chemistry*. 101: 267–273.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M. and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry and Cell Biology*, *39*(1): 44–84.

Woode, E., Alhassan, A. and Chrissie, S.A. (2011). Effect of ethanolic fruit extract of *Xylopia aethiopica* on reproductive function of male rats. *International Journal of Pharmaceutical and Biomedical Research*, 2(3): 161-165.

Zou, C.G., Agar, N.S. and Jones, G.L. (2001). Oxidative insult to human red blood cells induced by free radical iniator AAPH and its inhibition by a commercial antioxidant mixture. *Life Sciences*, 69(1): 75-86.