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Plasma oxidation and *in vitro* antioxidant activities of aqueous extracts of *Xylopia aethiopica* L. whole seed and pod

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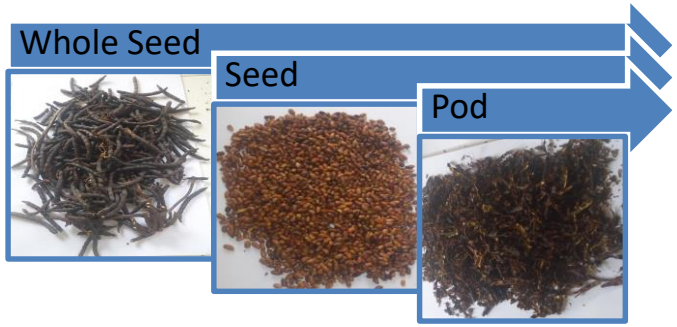
Graphical Abstract



Xylopia aethiopica L.
(*Annonaceae*)

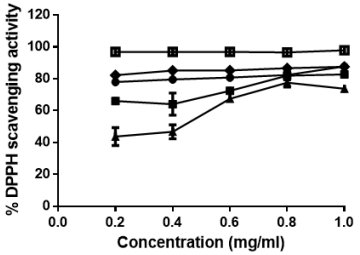
Plasma oxidation

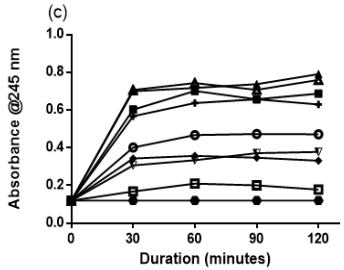
Erythrocyte protection

Thrombolytic activity

Membrane stability

In vitro antioxidant
DPPH, TAC, NO, H_2O_2





Abstract: In Nigeria, *Xylopia aethiopica* whole seed are locally used in the treatment of constipation. They are used as a tonic in tea and beverages, and after pregnancy delivery to facilitate the removal of clothing blood in the system. This study evaluated the plasma oxidation properties and *in vitro* antioxidant activities of *X. aethiopica* whole seed and pod. The DPPH 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 similar with 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±0.03mg/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. Protective effects of the extract of *X. aethiopica* were concentration dependent, with lower doses (6.25 – 25 µg/ml) having more protective effects against accumulation of $CuSO_4$-induced conjugated dienes in plasma. Similarly, the seed, pod and whole seed demonstrated significant ($p<0.05$) thrombolytic activity compared with normal control but not significantly compared with streptokinase. The present study demonstrated the protective nature of *X. aethiopica* seed, pod and whole seed extracts and recommended them for consideration as natural antioxidant source.

Keywords: *Xylopia aethiopica*; Antioxidant activities; Plasma; Oxidation; whole Seed; Pod

Introduction

Xylopiaa ethiopica L. (*Annonaceae*) is an evergreen, and aromatic tree that can grow up to 20m high. It is native to the lowland rainforests in the savanna zones of Africa (Orwa *et al,* 2009).

The fruits of *Xylopiaa ethiopica* are aromatic, quite pungent and slightly bitter. They are small twisted bean-like pods, dark-brown cylindrical, with 5-8 kidney shaped seeds.

In Nigeria, *Xylopia aethiopica* whole seed are locally used in the treatment of constipation. They are used as a tonic in tea and beverages, and after pregnancy delivery to facilitate the removal of clothing blood in the system.

The overall aim of the study was to evaluate the plasma protective effects and *in vitro* antioxidant properties of aqueous extracts of *Xylopia aethiopica* whole seed and pod to ascertain or otherwise its ethnopharmacological usage.

Materials and Methods

Chemicals: 2,2-diphenyl-1-picrylhydrazyl (DPPH), Hydrogen peroxide (H_2O_2), butylated hydroxytoluene (BHT), ascorbic acid, NaCl, Na_2HSO_4, NaH_2SO_4, triton X-100 was purchased from sigma chemical company, St. Luois, MO, USA. SIMCARD (Simvastatin) was purchased from Swiss Pharma, Gujarat, India. All other chemicals were of analytical grades and prepared using distilled water.

Plant Material: *Xylopia aethiopica* was obtained from Azare marked, Azare, Bauchi State Nigeria and identified at the Department of Botany, Bauchi state University by Mallam Umar Gadau. A voucher sample was deposited at the herbarium with the Federal College of Forestry, Jos, Plateau state.

Preparation of aqueous extract: The plant material was divided into three; seed, pod and whole seed (seed + pod). The aqueous extract of *xylopia aethiopica* was prepared by dissolving fifty grams (50 g) each of the powdered seed, pod and whole seed separately in 250 ml distilled water for 24 hours. After which the aqueous filtrate was further filtered using Whatman filter paper no. 1. The filtrates was concentrated using a rotary evaporator at 40^{o}C to generate the aqueous extracts respectively.

Materials and Methods cont.

Methods: The extracts scavenging activity against DPPH radical was determined according to the method described by McCune and Johns, 2002 while total antioxidant capacity (TAC) was evaluated using the phosphomolybdenum assay as described by Prieto et al., 1999, and nitric oxide (NO) scavenging capacity was measured according to the method of Fiorentino et al., 2008. Hydrogen peroxide activity was estimated using prepared samples in phosphate buffer saline as described by Ruch et al., 1989.

Plasma oxidation and erythrocyte protection were determined according to the methods described by Cheung et al., 2003 and Liao et al., 2014. Thrombolytic activity was done according to the methods prescribed by Prasad et al., 2007 while membrane stability was carried out according to Shinde et al., 1999.

Results and Discussion

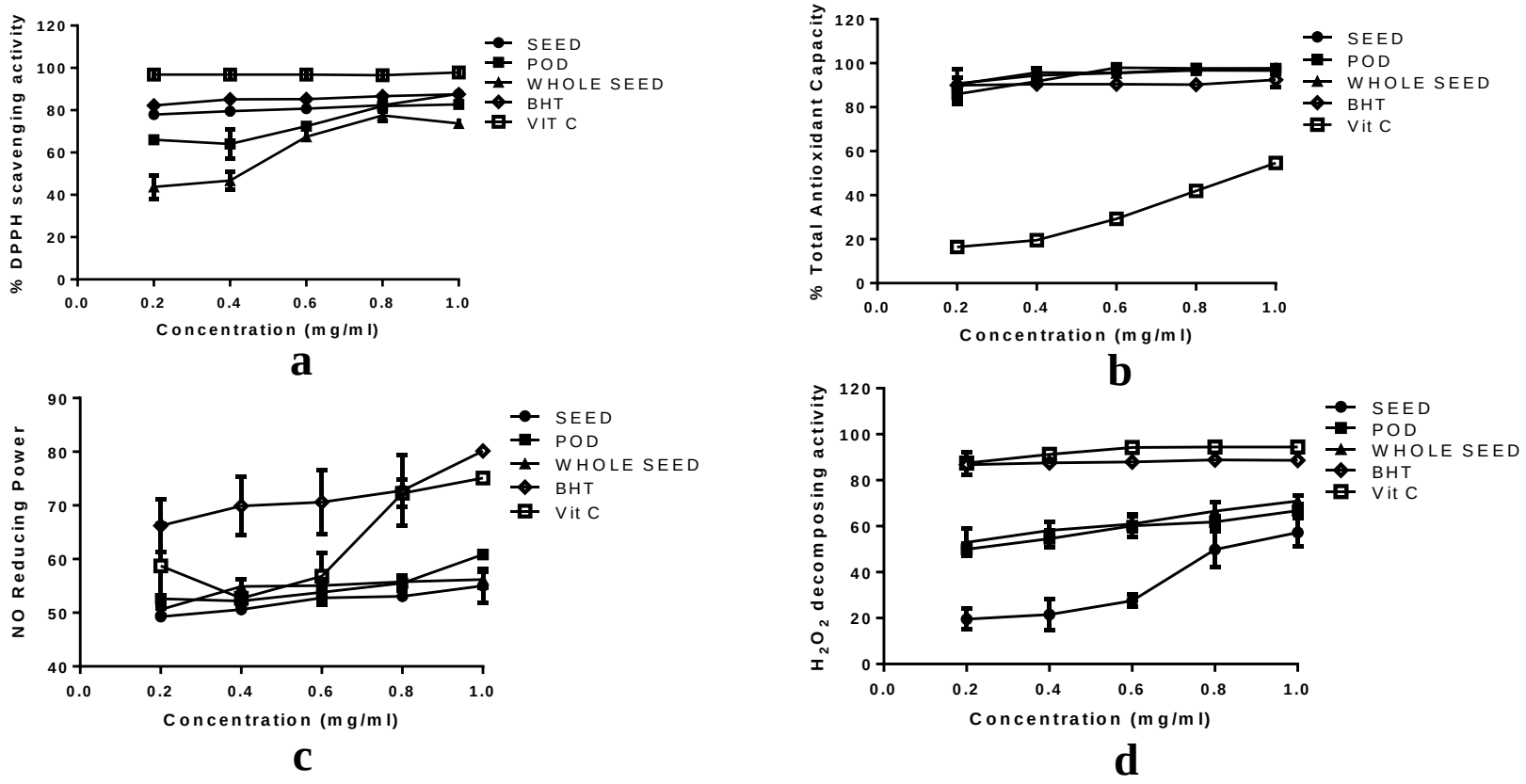


Figure 1: Antioxidant activities of aqueous extract of *Xylopia aethiopica*. (a) DPPH scavenging (b) Total antioxidant capacity (c) Nitric oxide reducing power (d) Hydrogen peroxide decomposing activities. Values are mean ± SEM of triplicate determinations. BHT = Butylated hydroxytoluene, Vit C = Ascorbic acid

Results and Discussion cont.

Table 1: IC_{50} values for various *in vitro* antioxidant activity of aqueous extract 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 mean ± 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.

Results and Discussion cont.

Table 2: Effects of aqueous extracts of *Xylopia aethiopica* on erythrocyte lysis

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 mean of triplicated determinations

Results and Discussion cont.

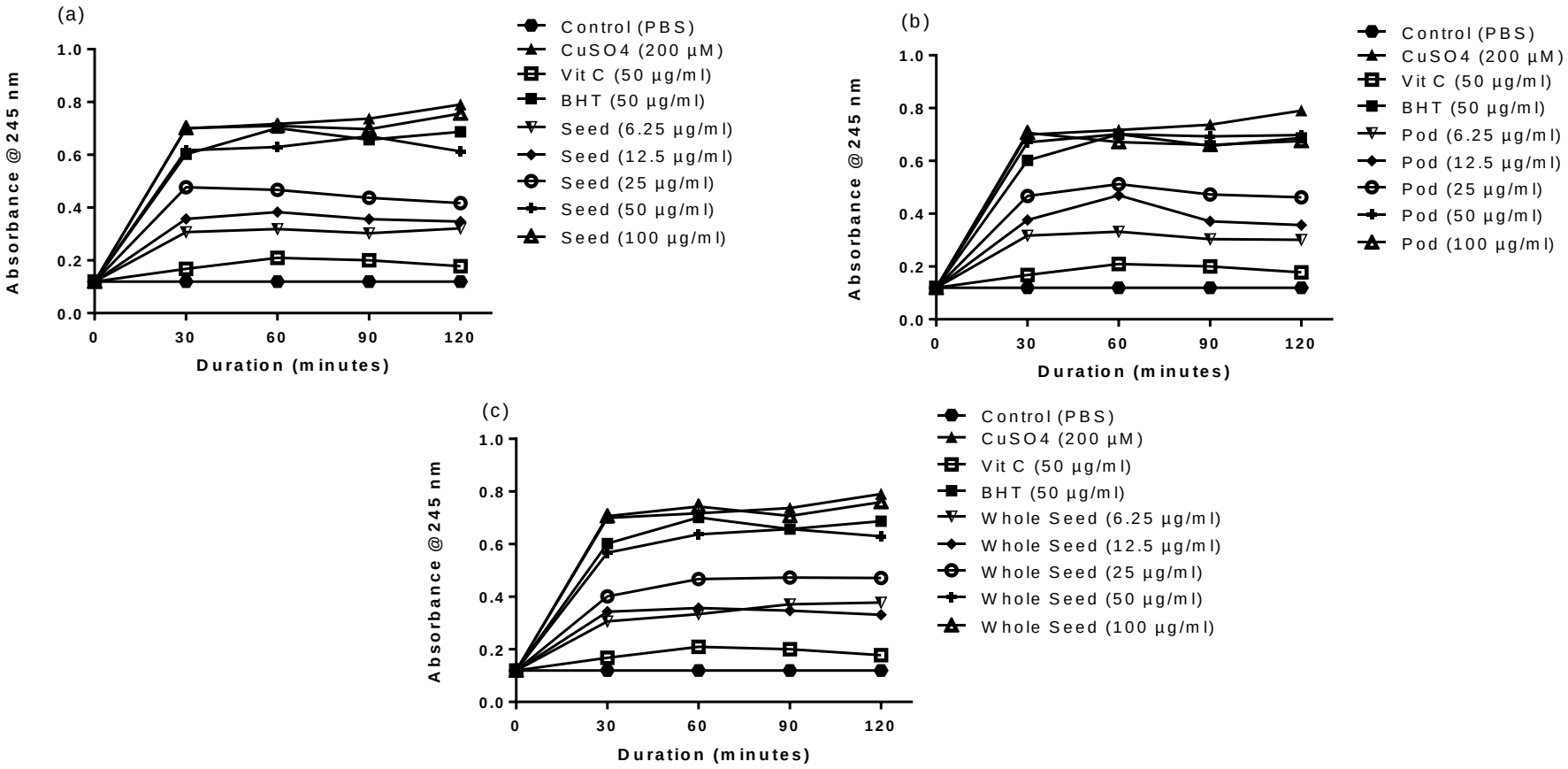


Figure 2: Protective effects of aqueous extracts of *Xylopia aethiopica* against $CuSO_4$-induced accumulation of conjugated dienes in plasma. (a) seed, (b) pod and (c) whole seed, Values are mean ± SEM of three determinations

Results and Discussion cont.

Table 3: Erythrocytes protection effects of aqueous extracts of *Xylopia aethiopica*

Samples	% Inhibition
Control (PBS)	66.94
Seed (6.25 µg/ml)	65.31
Seed (12.5 µg/ml)	53.06
Seed (25 µg/ml)	71.02
Seed (50 µg/ml)	64.49
Seed (100 µg/ml)	30.20
Pod (6.25 µg/ml)	76.33
Pod (12.5 µg/ml)	48.98
Pod (25 µg/ml)	67.76
Pod (50 µg/ml)	61.63
Pod (100 µg/ml)	77.14
Whole Seed (6.25 µg/ml)	55.92
Whole Seed (12.5 µg/ml)	59.18
Whole Seed (25 µg/ml)	58.78
Whole Seed (50 µg/ml)	48.98
Whole Seed (100 µg/ml)	20.41
Butylated hydroxytoluene (100 µg/ml)	47.76
Ascorbic acid (100 µg/ml)	46.12

Results and Discussion cont.

Table 4: Thrombolytic activity of aqueous extracts of *Xylopia aethiopica*

Test groups	% Thrombolysis
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

Results and Discussion cont.

Table 5: 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

Conclusions

The present study demonstrated that *Xylopia aethiopica* L. seed, pod and whole seed:

1. possesses significant *in vitro* antioxidant properties.
2. radical scavenging activities (IC_{50}) were comparable with the reference compounds (vitamin C and Butylated hydroxytoluene).
3. do not posses RBC membrane lyses at the doses used in the study.
4. does not have negative effect on erythrocyte hemolysis.

They are recommended for consideration as natural antioxidant source and for development of phytomedicine.

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