Research Article

Phytochemical composition, *In vitro* antioxidant capabilities and Immunomodulatory indices of *Xylopia aethiopica* fruit extracts

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Abstract

Background: Recently, there is increased interest in the scientific investigations of botanicals with immunomodulatory capabilities due to the alleged efficacy in achieving quality health. *Xylopia aethiopica* fruit is a popular recipe employed in brewing of soup/broth in the southern region of Nigeria and some parts of Africa. **Objective**: This study determined the effect of solvent partitioning on the phyto-constituents, *in vitro* antioxidative activities and immunomodulatory potentials of *Xylopia aethiopica* fruit extracts (crude ethanolic extract, aqueous and n-hexane partitioned extracts). **Materials and methods**: The phyto-constituents in the extracts were determined by colorimetric test, while *in vitro* antioxidant activities were assessed by the radical scavenging abilities, metal ion chelating potential and reducing potential of the extracts and the immunomodulatory potentials were evaluated by the carbon clearance capability, avidity of neutrophil, inhibition of cyclophosphamide induced neutropenia and zinc sulphate turbidity test. The extracts were tested at doses of 100 and 200 mg/kg body weight and levamisole was used as the standard drug.**Results**: Various phyto-constituents such as and terpenoids indicated marked reactions and recorded increases ($p<0.05$) in the crude ethanol extract than the partitioned extracts. Consequentially, the crude ethanol extract presented the least EC_{50} of the DPPH radical scavenging activity and metal ion chelating potential. In the same vein, the crude ethanol extract, as well as levamisole increased the adhesion of neutrophils in the blood to nylon fibre ($p<0.05$). The phagocytic index recorded increases ($p<0.05$) in the fruit extracts. Cyclophosphamide induced neutropenia was inhibited ($p<0.05$) by the standard drug and all the extracts of *X. aethiopica*. **Conclusion**: The results indicated that the crude ethanol extract exhibited most impressive antioxidant activity and also, improved immunoactivities than the levamisole and partitioned extracts. Therefore, the consumption of *X. aethiopica* fruit in soup/broth brewing, as well as alcoholic tincture is recommended for improved quality life.

Keywords: Antioxidant activity, Immunomodulation, Improved quality life, Phyto-constituents, Phagocytic index, *Xylopia aethiopica*

Introduction

Modulation of body's integrated response to an antigen through stimulation or suppression has been suggested to be effective in maintaining a disease free state (Bafna and Mishra, 2005). Immunomodulation is a very complex phenomenon implicated in all disease and health states. Immune disorders have been linked to the genesis of many immuno-pathological disorders such as autoimmune pathologies and immunodeficiency diseases. Several synthetic drugs have been used to normalize or modulate these pathophysiological processes; however, many of these therapeutic options are limited either as a result of rapid development of resistance, their cost or their reported adverse effects such as depression, fatigue, sleepiness, loss of appetite, neutrophilia, lymphopenias, and increase antibiotic-resistant strains of microorganisms (Srisilam, 2000; Wichers and Maes, 2004). Considering these limitations with the synthetic therapy, it could prove

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helpful to consider the use of phytotherapy in the modulation of immune responses; as immune-related illnesses have long been treated with herbs (Azeb et al., 2004; Motrin, 2005).

Recently, attention is been focused on the use of botanicals in the modulation of the immune system towards achieving a healthy status to ward off opportunistic infections (Oyewo et al., 2013). *Xylopia aethiopica* fruit has been used throughout history for both culinary in special local preparations of "pepper soup" and "kunnu" in the Southwestern parts of Nigeria, and medicinal purposes to treat bronchitis, asthma, infertility, wounds, arthritis and rheumatism, post-natal pains and dysenteric conditions among many others (Burkill 1985; Fall et al*.,* 2003; Ogunkunle and Ladejobi, 2006; Ezekwesili et al*.,* 2010). It is native to the lowland rainforest and moist fringe forest in the savanna zones of Africa, but largely located in West, Central and Southern Africa (Tairu et al., 1999). The fruit is thought to possess pharmacologically active compounds which certainly play important role in human health. Most of these compounds have been shown to down regulates many degenerative processes and diminish the severity of disease, particularly those in which inflammation plays a role (Oso et al*.,* 2017).

The therapeutic value of *Xylopia aethiopica* fruit should be inherent in the phyto-constituents that could produce definite physiological and pharmacological actions. Some of the renowned phyto-constituents with scientific evidences to suppress the inflammatory processes, moderate cell signalling pathways, proliferation, apoptosis, redox balance and most often appear to be protective against cancer, neurodegenerative disorders and cardiovascular diseases are alkaloids, tannins, terpenoids, flavonoids and phenolic compounds (Aggarwal et al., 2006; Rahman, 2007; Oyewo et al., 2013). However, various studies have shown that the efficacy of the extraction yields and the antioxidant activities depend on the nature of the solvent used for the extraction of the active principles (Antolovich et al., 2000; Delfanian et al., 2015). Thus, the present study is aimed at evaluating the influence of solvent partitioning on the phytochemical composition antioxidant and immunomodulatory properties of ethanol extract of *Xylopia aethiopica* fruit.

Materials and methods

Plant materials

The fruit of *Xylopia aethiopica* was collected from farms in the North Central Zone of Kwara State. It was authenticated at the Department of Plant Biology, University of Ilorin with the voucher number UIH001/1089.

Experimental animals

Adult male Wistar rats that weighed 265.42 ± 11.37 g were sourced from the breeding unit of experimental animal holding facility in the Department of Biochemistry, University of Ilorin, Nigeria. They were housed in plastic cages with steel mesh in the research unit of experimental animal holding facility in the Department of Biochemistry, University of Ilorin, Nigeria.

Chemicals and reagents

All chemical were of analytical grade. Gallic acid, trolox, linalool, ferrozine, catechin, 1, 1-diphenyl-2-picrylhydrazyl, were products of Sigma Chemical Co. (St. Louis, MO, USA). Potassium ferricyanide, epinephrine, acetic acid, Folin Ciocalteu's phenol reagent were products of Loba Chemie, India, while hydrochloric acid, sulphuric acid, ethanol, n-hexane were products of Guangdong Guangnua, China.

Preparation of plant extracts

The fruit of *Xylopia aethiopica* was air-dried in the shade at room temperature (30 ± 2 °C) till when a mere finger squeezing could break the epicarp. The dried fruits were pulverized using a domestic grinder, stored in air-tight containers and kept away from sunlight. The pulverized fruit of *X. aethiopica* was mixed with ethanol for seventy-two hours and solubilised with the aid of a shaker. The solution was filtered with Whatman paper 4 and the extractant (crude ethanolic extract (ECE)) was dried by evaporation under vacuum (rota-evaporation). The resulting extract was further partitioned into aqueous fraction (APE) and n-hexane fraction (HPE), respectively.

Phytochemical analyses

The qualitative phytochemicals screening was performed to determine the biologically active compounds based on various functional groups present in the dried fruit, using standard methods as described by Sofowora (1993) and Trease and Evans (2005), while the phytochemicals were determined quantitatively for total phenolics (Singleton et al., 1999), total flavonoids (Zhishen et al., 1999), terpenoids (Ghorai et al. 2012), flavonol (Abdel-Hameed, 2009), stearoids (Okeke and Elekwa, 2003), saponins (Obadoni and Ochuko, 2001) and tannins (Chang et al*.,* 1994).

Assessment of *in vitro* antioxidant activity

The total antioxidant capacity was determined according to the method described by Prieto et al. (1999) with slight modifications. This is based on the reduction of Mo (VI) to Mo (V) by the antioxidant agents and the subsequent formation of a green phosphate / Mo (V) complex with a maximal absorption at 695 nm. Briefly, 300 µl of the sample extract was mixed with 3ml of the working reagent (28mM Na_2HPO_4, 4 mM $(NH_4)_2MoO_4$, 0.6M H_2SO_4). The reaction

mixture was incubated for 90 minutes and the absorbance was read at 695nm. The total antioxidant capacity was calculated using gallic acid as standard and expressed in mg/g of the extract from the standard curve (y=0.010, x=0.107 and R^2=0.996).

The ferric reducing antioxidant power, DPPH (1, 1-diphenyl-2-picryhydrazyl) scavenging activity and metal ion chelating potential were determined respectively by standard methods described by Oyaizu (1986), Gyamfi et al. (1999) and Dinis et al. (1994).

Experimental design and animal handling

The rats were randomized into two sets 'A' and 'B' and allowed free access to standard rat chow and water. They were maintained at room temperature of 30 ± 2°C and light (12 hours day: 12 hours night), and were allowed 14 days to acclimatize. Set 'A' were picked into eight (8) groups, comprised of ten rats each, while set 'B' comprised of four rats in nine groups.

A working solution of each extract was prepared by dissolving separately calculated weights of each of the extract in 5% dimethylsulfoxide (DMSO) and then in distilled water to make doses of 100 and 200 mg/kg body weight of the rats. The various extracts were administered orally once daily at 17:00 hrs ± 30 minutes, GMT.

The set 'A' rats were handled such that: control, group 1 received 5% DMSO v/v of distilled water, group 2 received the standard drug (levamisole or diclofenac at 2.5 mg/kg body weight), groups 3 and 4 received ethanolic extract at 100 and 200 mg/kg body weight respectively, groups 5 and 6 received aqueous partitioned extract at 100 and 200 mg/kg body weight and groups 7 and 8 received n-hexane partitioned extract at 100 and 200 mg/kg body weight.

The set 'B' rats were used for the assessment of the protective effects of the various extracts against cyclophosphamide induced neutropenia by administering the same doses as reported in set A. However, there were two control groups in set B rats, one none induced control and the other induced control. The standard drug solutions were prepared in distilled water and were administered orally.

All experiments were performed in accordance with ethical laws and with the approval of the University of Ilorin Ethical Review Committee with UERC approval number UERC/ASN/2015/047.

Immunomodulatory indices

Neutrophil adhesion test

The neutrophil adhesion test was carried out according to the method described by Fulzele et al. (2003). The rats in different groups received the extracts, orally for fourteen days. Blood samples were collected through cardiac puncture into heparinised vials from fasted animals in all the groups, sixteen hours after the last administration under mild diethylether anaesthesia. The differential leukocyte counts (DLC) were determined after fixing the blood smear and staining with the Giemsa's stain. After the initial counts, blood samples were incubated with 80 mg/ml of nylon fibres for 10 minutes at 37 °C. The incubated blood samples were again analysed for DLC. The difference in the neutrophil count before and after incubation of blood sample with nylon fibres was determined.

Carbon clearance test

The carbon clearance test was carried out according to the method described by Gokhale et al. (2003) with slight modification. The rats were administered the extracts orally for five days. Forty-eight hours after the last dose of the extracts, the rats were injected with 0.1 ml of carbon suspension ink via the tail vein. Blood samples were withdrawn at five minutes and fifteen minutes. A 50 µl blood sample was mixed with 4 ml of 0.1 % sodium carbonate solution and absorbance was read at 660 nm.

Phagocytic index K is calculated using the equation:

$$K = (Log_e A_1 - Log_e A_2) / (t_2 - t_1)$$

Where, A_1 and A_2 were the absorbance values at five and fifteen minutes (t_1 and t_2) respectively.

Zinc sulphate turbidity test

The zinc sulphate turbidity test was carried out according to the method described by Pfeiffer et al. (1977). This is a semi-quantitative test for plasma globulin and it is based on precipitation of globulin by low concentrations of metal ions and subsequent turbidometric analysis. Plasma (100 µl) from the blood samples of the animals was mixed with 6 ml of $ZnSO_4$ which was prepared by dissolving 208 mg of $ZnSO_4$ in 1 litre of distilled water that had been boiled to remove the dissolved carbon dioxide. The mixture was incubated at 29°C for one hour and the turbidity was measured using spectrophotometer at 490 nm. The values were reported as percentage transmittance.

Protective effect against cyclophosphamide-induced neutropenia

The protective effect of the extract against cyclophosphamide induced-neutropenia was carried out according to the method described by Jayathirtha and Mishra, (2004) with slight modifications. The rats were administered the extracts orally for ten days. On the tenth day, neutropenia was induced by injecting the rats with cyclophosphamide (200 mg/kg body weight rats) intra-

peritoneally (Thatte et al., 1987). Blood samples were collected through retro-orbital vein three days before the induction and three days after, and total leukocyte count (TLC) and differential leukocyte count (DLC) were estimated to ascertain successful induction of neutropenia. The oral administration continued till sixth day after the induction and the TLC and DLC were also estimated. The two control groups received physiological saline.

Statistical Analysis

All data are reported as mean ± standard error, and analysed using one way analysis of variance (ANOVA) with a Fisher's Least Significant Difference post hoc test to determine significant differences ($p<0.05$) between groups.

Results

Phytochemical analyses and *In vitro* antioxidant capabilities

The results of the phytochemical composition of the ethanol fruit extracts of *X. aethiopica* and the partitioned extracts are presented in Table 1. The ethanol extract showed highest phenolic (982.50 ± 28.10 mgGAE/100g), flavonoid (424.00 ± 7.75 mgCE/100g) and saponin (mg/100g) contents. Flavonoids were absent in the n-hexane partitioned extract. There was no significant difference between the total flavonoids, flavonol and tannins in the ethanol extract and the aqueous partitioned extract, at $P<0.05$, suggesting that the n-hexane partitioning did not have significant effect on the contents of these compounds. Conversely, the values of the total phenolics, saponins and sterols differ significantly among the extract. The terpenoids (458.76 ± 48.04 mgLE/100g) and phytosterol (93.05 ± 1.86 mgChE/100g) levels were found to be highest in the n-hexane partitioned extract.

Table1. Phytochemicals components of ethanol extract and the partitioned extracts of the fruits of *X. aethiopica*

Parameters	ECE	APE	HPE
Total PhenolicsmgGAE/100g)	982.50±28.10^a	818.07±7.30^c	329.17±12.02^b
Total Flavonoids (mgCE/100g)	424.00±7.75^a	407.83±3.25^a	-
Terpenoids (mg LE/100g)	280.08±48.04^a	-	458.76±36.45^b
Flavonols (mg CE /100g)	40.11±1.66^a	42.38±6.08^a	18.91±0.75^b
Saponins (mg/100g)	42.42±2.28^a	30.67±1.17^c	14.48±0.86^b
Phytosterols (ChEmg/100g)	51.71±4.37^a	38.77±0.83^c	93.05±1.86^b
Tannins (mgGAE/100g)	56.56±1.44^a	46.16±2.89^a	-

Values are expressed as mean of triplicate determinations ±standard error of mean. Different superscripts across the row indicate significant differences at $P<0.05$.ECE=Ethanol Extract, HPE=n-hexane partitioned extract, APE=Aqueous partitioned extract, CE= Catechin Equivalent; GAE= Gallic Acid Equivalent; ChE=Cholesterol Equivalent; LE=Linalool Equivalent

The antioxidant properties were assessed by various methods such as estimation of total antioxidant capacity, ferric ion reducing potential, metal chelating activity and 1,1-diphenyl-2 picrylhydrazyl (DPPH*) scavenging activity. The results of the antioxidant properties are shown in Table 2. The ethanol crude extract of *X. aethiopica* fruit was found to have the lowest EC_{50} value of the DPPH* scavenging activity (90 µg/ml) followed by the aqueous partitioned extract (300 µg/ml). The EC_{50} value of the metal chelating potential was also found to be lowest in the ethanol crude extract (390 µg/ml) followed by the aqueous partitioned extract (410 µg/ml). The reference standards used for the scavenging activity and chelating activity were Trolox (EC_{50}: 60 µg/ml) and EDTA (EC_{50}: 160 µg/ml), respectively. The ethanol crude extract had the highest amount of total antioxidant expressed as mg/g of gallic acid equivalent followed by the aqueous partitioned extract, and the highest ferric ion reducing potential expressed as mg/g of ascorbic acid equivalent followed by the n-hexane partitioned extract.

Table 2. Antioxidant properties of ethanol extract and partitioned extracts of *X. aethiopica*

Samples	TAC (mg/g)	FRAP (mg/g)	EC_{50} (µg/ml)	
			DPPH*	MIC
ECE	59.16±0.16^a	29.09±0.44^a	90.0^a	390^a
APE	35.76±0.12^c	7.08±0.29^c	300^b	410^b
HPE	30.08±1.46^b	12.05±0.35^b	325^c	440^c
Trolox	-	-	60^d	-
EDTA	-	-	-	160^d

EC_{50} values were calculated from the slope equations of the dose-response curves Different superscripts within a column indicate significant differences at $P<0.05$. HPE=n-hexane partitioned extract, APE=Aqueous partitioned extract, ECE=Ethanol Extract, TAC= Total Antioxidant Capacity, FRAP=Ferric reduction antioxidant potential DPPH=1, 1-diphenyl-2-picryhydrazyl Scavenging Activity, MIC=Metal Ion Chelating Assay, EDTA = Ethylenediaminetetraacetic acid.

Immunomodulatory indices

The results of the immunomodulatory capabilities of the ethanolic extract, n-hexane and aqueous partitioned extracts in Wistar rats are presented in Tables 3, 4 and 5. The adhesion ability of neutrophils was increased ($p<0.05$) in the rats administered with the crude ethanolic extract and levamisole. Surprisingly, significant decreases ($p<0.05$) were recorded in the rats that received the partitioned extracts and 200 mg/kg body weight dose of the crude ethanolic extract compared to 100 mg/kg body weight dose (Table 3). In Table 4, increases ($p<0.05$) were obtained in the phagocytic index in rats that received ethanolic extract and aqueous partitioned extracts both at 200 mg/kg body weight dose only. However, a rather inconsistent pattern was obtained in the immunoglobulin levels that were either not significant ($p>0.05$) at 100 mg/kg body weight dose or reduced ($p<0.05$) at both doses in all the rats that received the fruit extracts of *X. aethiopica* (Table 4). The fruit

Table 3. Immunostimulant activity of ethanolic extract, aqueous and n-hexane partitioned extracts of *X. aethiopica* by chemotaxis

Groups	Neutrophil (%)		Percentage Reduction
	Before Incubation	After Incubation	
Control	21.50±1.33	20.10±0.91	1.40 ± 0.36^{a}
HPE100mg/kg	21.93±0.66	20.17±0.93	1.77 ± 0.39^{ab}
HPE200mg/kg	32.73±2.84	31.67±2.98	1.07 ± 0.24^{d}
ECE100mg/kg	28.97±2.89	23.87±2.09	5.10 ± 2.67^{c}
ECE200mg/kg	28.43±2.65	26.53±2.51	1.90 ± 0.29^{b}
APE100mg/kg	22.13±2.36	20.93±2.07	1.20 ± 0.45^{ad}
APE200mg/kg	37.93±2.47	36.77±2.31	1.17 ± 0.17^{d}
Lev	17.07±1.74	11.77±2.98	5.30 ± 1.27^{c}

Data presented as Mean ±SEM, n=6.Different superscripts down the column indicate statistically significant differences at $p<0.05$.HPE=n-hexane partitioned extract, ECE=Ethanolic extract, APE=Aqueous partitioned extract, Lev=Levamisole

Table 4. Phagocytic index and concentration of plasma immunoglobulin of rats administered with ethanolic extract, aqueous and n-hexane partitioned extracts of *X. aethiopica*

Groups	Phagocytic index ($\times 10^2$)	Plasma immunoglobulin levels (%Transmittance)
Control	3.96 ± 0.50^{a}	48.75 ± 6.13^{a}
HPE100mg/kg	2.18 ± 0.20^{e}	41.07 ± 2.92^{c}
HPE200mg/kg	2.05 ± 0.20^{e}	37.78 ± 2.05^{cd}
ECE100mg/kg	3.42 ± 0.30^{ad}	53.55 ± 0.66^{a}
ECE200mg/kg	5.13 ± 0.10^{b}	42.07 ± 1.40^{ac}
APE100mg/kg	3.54 ± 0.10^{ad}	50.43 ± 3.14^{a}
APE200mg/kg	5.93 ± 0.10^{c}	35.13 ± 3.18^{d}
Lev	3.54 ± 0.10^{d}	66.82 ± 4.08^{b}

Data presented as Mean±SEM,n=6, . Different superscripts within a column indicate significant differences at $p<0.05$.HPE=n-hexane partitioned extract, ECE=Ethanolic extract, APE=Aqueous partitioned extract, Lev=Levamisole

extracts of *X. aethiopica* recorded marked recuperation ($p<0.05$) at 3 and 6 days post neutropenia induction than the standard drug, levamisole (Table 5).

Discussion

The therapeutic potentials of all plants are renowned to be inherent in the secondary metabolites (phytochemicals) that are responsible for eliciting varied and definite physiological action in mammals. In Table 1, the depicted concentrations of the various phytochemical in the fruit extracts of *X. aethiopica* indicated that the solubility of the phytochemicals is solvent dependent and also, many of the phytochemicals are partitioned into the aqueous medium than the non-polar n-hexane medium. The findings agree with the studies of Ayinde et al. (2007) and Tijani et al. (2009) that not all phytochemicals present in parts of plant could be extracted completely and that the type of the extracting solvent determine the extent of extraction. Therefore, the variations recorded in the partitioned extracts and crude extract of *X. aethiopica* fruit (Table 1) could be adduced to solubility incompatibilities of the functional groups in the phytochemicals and extracting solvents. However, the quantified phytochemicals in the fruit extracts of *X. aethiopica* are renowned for the various biological effects, such as antioxidative, anti-inflammatory, antimicrobial, membrane labialization, enzyme induction, immunomodulatory amongst others.

The antioxidative capacities of the fruit extracts presented in Table 2 indicated that the ethanol crude extract had the best antioxidative capability than the partitioned extracts. This is due to likely insolubility of some phytochemicals that led to the loss of synergistic actions among the phytochemicals in the partitioned extracts. The trend is supported slightly by the previous study of Hamberger and Hatettman (1991), which reported that crude extracts are biologically more active than their isolated active principles, possibly due to the combined effects of various components present in the crude extract or inability of some solvent to extract the active components. Our study revealed that the solvent partitioning reduced the antioxidant potential of the crude extract largely, except in the ferric reducing antioxidant potential. However, the aqueous partitioned extract had higher antioxidant potential than the n-hexane partitioned extracts: suggesting that the factors that contribute to the antioxidant properties of the fruit are perhaps mostly hydrophilic. This assertion is strongly supported by the absence of flavonoid compounds, which are hydrophilic, in the n-hexane partitioned extract. Therefore, it is logical to infer that the antioxidant activity of the fruit of *X. aethiopica* could be a direct correlation of the flavonoid contents.

The beneficial medicinal effects (anti-oxidative and ainti-

Table 5. Protective effect of ethanolic extract, aqueous and n-hexane partitioned extracts of *X. aethiopica* in neutropenic rats

Groups	3 days before	3 days after	% Reduction	6 days after	Reduction
Co	25.17±1.17[a]	25.00±0.89[a]	0.68	25.00±1.27[a]	0.68
Ci	24.67±1.75[a]	14.83±0.98	39.87	15.50 ± 1.64[c]	37.17
HPE100mg/kg	26.00±0.63[a]	16.06±1.63[b]	38.23	20.69 ± 1.41[ab]	20.42
HPE200mg/kg	25.83±0.75[a]	15.84±1.37	38.68	21.51 ± 1.76[ab]	20.60
ECE100mg/kg	27.90 ± 0.55[b]	16.41±1.87[b]	27.08	24.07 ± 1.51[a]	8.49
ECE200mg/kg	26.87 ± 0.75[ab]	16.21±1.21[b]	31.06	22.82 ± 1.21[ab]	11.44
APE100mg/kg	27.00 ± 0.89[ab]	16.10±1.51[b]	38.08	21.65 ± 1.27[ab]	14.58
APE200mg/kg	25.83 ± 1.33[a]	16.00±2.10[b]	38.06	20.45 ± 1.63[b]	18.83
Lev	26.17 ± 0.75[ab]	15.5 ± 1.05[c]	38.42	20.00 ± 1.79[b]	20.54

Data presented as Mean ±SEM, n=6, Different superscripts within a row indicate significant differences at $p<0.05$.HPE=n-hexane partitioned extract, ECE=Ethanolic extract, APE=Aqueous partitioned extract, Lev=Levamisole Co=Control, Ci=Induced control.

inflammatory) of plants are often attributed to the antioxidant activities of the phytocompounds mainly the phenolics, flavonoids and flavanol (Miliauskas et al*.,* 2004). In addition, prior scientific evidence suggests that saponins exert some beneficial effects which include lowering of cholesterol level and stimulation of immune system (Oyewo et al., 2013).

The immunomodulatory activities of the crude extract and the partitioned extracts were investigated in Wistar rats using different models such as neutrophil adhesion test, carbon clearance test, zinc sulphate turbidity test, protection against induced neutropenia. The neutrophil adhesion test was carried out to evaluate the immuno-stimulant activity of the test sample by chemotaxis, which would be a reflection of the ability or readiness of the recruitment of cells (neutrophils) in the blood vessels to the site of inflammation. The result obtained in Table 3 demonstrated that the crude ethanolic extract at 100 mg/kg and levamisole increased the avidity of neutrophils in the blood to nylon fibre. This adherence, as hypothesised by Hajra et al.(2012), could be due to the increased expression of β_2 integrins (CD_{18}). Although, β_2 integrins was not determined in our study, it is a glycoprotein that is exclusively expressed on the surface of leukocytes, mainly the innate cells and mediates the adherence of the cells to extracellular matrix proteins of the basement membrane or to ligands on other cells (Parkin and Cohe, 2001; Kasper et al*.,* 2007). This could be responsible for the enhanced avidity of the neutrophils to the nylon fibres in the fruit extracts of *X. aethiopica* and the standard drug. It is worthy of note that the administration of the fruit extracts of *X. aethiopica* at lower concentrations presented better avidity of the neutrophils to the nylon fibre than at higher doses. Thus, the loss in avidity could likely be due to overstimulation of neutrophils secretion and binding by the fruit extracts of *X. aethiopica* that might have led to their selective destruction (hyper- active neutrophils) during maturation by the immune cell regulatory mechanism.

The carbon clearance assay, also known as the phagocytic index, is the rate of clearance of colloidal carbon, injected intravenously into the systemic circulation, from the blood. It is used to assess the effect on reticuloendothelial cell mediated phagocytosis (Jayathirtha and Mishra, 2004; Oyewo et al., 2017). Reticuloendothelial cell comprises of fixed and mobile macrophages and they are important in the clearance of microorganisms, malignant cells, tissues debris and inorganic substances (Ghule et al*.,* 2006). The trends in phagocytic index in rats administered the fruit extracts of *X. aethiopica* at 100 and 200 mg/kg body weight of crude ethanolic and aqueous partitioned extract showcased a boost in immune functions (Table 4). Thus, the crude extract and aqueous partitioned extract would stimulate the reticuloendothelial system to combat or ward off infection and diseases, reflecting the probable consequence of *X. aethiopica* on phagocytic function of mononuclear macrophages. Our results in the phagocytic indexes are similar to those reported by Ismail and Asad (2009) using the extracts of *Ocimum sanctum* and *Acacia catechu*. However, the patterns reported at 200 mg/kg body weight of crude ethanolic and aqueous partitioned extract of *X. aethiopica* supported the suspected overstimulation of the secretion and binding of innate immune cells.

Zinc sulphate turbidity test is a semi-quantitative test for immunoglobulin in the plasma based on precipitation by low concentration of metal ions and subsequent turbidometric analysis. It is a direct measure to detect the humoral immunity (Pfeiffer et al*.,* 1977), especially the antibodies produced by the B-cells and plasma cells, which are central to immune response. In Table 4, the secretion of immunoglobulins were not stimulated and, or the enhancement of the functional properties of the B-cells. The partitioned fruit extract of *X. aethiopica* at 200 mg/kg body weight inhibited immunoglobulin secretions or activated their binding and consequential destruction or removal.

This might require further investigations.

Cyclophosphamide is an alkylating compound commonly used as an antineoplastic or cytotoxic chemotherapy drug due to its capability to meddle with DNA synthesis and subsequently its pharmacological action on cell proliferation (Ben-Efraim, 2001). It has been used expansively as immunosuppressant (Ismail and Asad, 2009), for the treatment of inflammatory disorders, prevention of autoimmune responses and promotion of tolerance (during post-transplantation) in alloreactive host; possibly due to its ability to induce myelo-suppression. In the present study, the induced neutropenia were inhibited by the standard drug, levamisole, and the extracts compared to the negative control (induced but not treated) (Table 5). The trends indicated that the fruit extracts of *X. aethiopica* may have stimulatory effects on the haematopoietic system, possibly through the secretion of immune cells and enhanced avidity. This must have involved cytokines that are involved in the regulation of immune secretion, chemotaxis and binding of immune cells. Oso et al. (2017) reported the anti-inflammatory role the soluble mediators of immune systems such as cytokines, due to likely insolubility of some phytochemicals that led to the loss of synergistic actions among the phytochemicals in the partitioned extracts.

On the foregoing, the crude extract and aqueous partitioned extract of *X. aethiopica* fruit have indicated favourable immunomodulatory properties, which are in conformity with preceded works reported by various authors on the favourable immunomodulatory characteristics of botanicals such as *Curcuma longa* (Chan, 1995), *Harpagophytum procumbens* (Fiebich et al*.,* 2001), *Zingiber officinale* (Chang et al*.,* 1995), *Caesalpinia bonducella* (Shukla et al*.,* 2009) *Syzygium aromaticum*, (Carrasco et al*.,* 2009) and *Allium sativum* (Clement et al*.,* 2010). The favourable immunomodulatory properties might be adduced to the level of saponins obtained in the crude extract and aqueous partitioned extract (Table 1). In addition, the recorded trends in the total phenolics, total flavonoids and flavonols, as well as the indicated antioxidative capabilities of the extracts must be the underlining factors in the reported immunomodulatory properties. This is not farfetched as Ismail and Asad (2009), Oyewo et al. (2017), Oso et al. (2017): all reported the roles of oxidative stress and inflammatory response in cytokines mediated immune modulation in health and diseases.

Conclusion

The overall results of the present study indicated that the partitioned extracts of *Xylopia aethiopica* fruits was not reliable, while the crude ethanolic extract of *Xylopia aethiopica* possessed the highest trends in the isolated phytochemicals, antioxidative capabilities as well as the favourable modulation of the immune cells through the stimulation of the cell mediated immunity, but not the humoral immunity. Therefore, the consumption of *Xylopia aethiopica* fruit in soup/broth brewing, as well as alcoholic tincture is recommended for improved life quality.

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