Nutrient, bioactive components and effects of ethanol extracts of the leaves *Annona muricata* and roots of *Fagara zanthoxyloide* on zidovudine-induced oxidative stress in wistar rats

ABSTRACT

The study was designed to determine the nutrient, bioactive components and the effects of ethanol extracts of the leaves *Annona muricata* and the roots of *Fagara zanthoxyloide* on zidovudine-induced oxidative stress in wistar albino rats. Wistar albino rats were divided into four (4) groups of five (5) rats each. Groups 2-4 were induced with 100g/ml/Kg bw of zidovudine and varying concentrations of the extracts (group 3 and 4); while group 1 served as the control. The results of the proximate composition of both plants showed the following ranges: moisture (10.32-18.30%), ash (0.65-9.45mg/g), crude protein (1.38-10.54mg/g), crude fat (2.35-9.73g/mg), crude fibre (3.00-15.53mg/g) and carbohydrate (50.19-65.23%). Iron, zinc and copper as well as folate and ascorbic acid were the highest minerals and vitamins present in both samples. Phytochemical composition results revealed higher concentrations of alkaloids, flavonoid and phenols in the leaves and roots of both samples. Acute toxicity study revealed no short term toxicity below 6g/ml/Kg bw for the leave extract of *Annona muricata* and 4g/ml/Kg bw for the root extract of *Fagara zanthoxyloide*. Administration of zidovudine to albino rats resulted in a significant increase ($p \leq 0.05$) in biomarkers of oxidative stress; while subsequent treatment with extracts of the leaves of *Annona muricata* and roots of *Fagara zanthoxyloide* resulted in decreases in the activities of superoxide dismutase, catalase and glutathione. The splenic histology revealed athropy, early-onset necrosis and reduction in sinusoidal pore size in the negative control group which were absent in the extract treatment groups which showed preserved splenic architecture with no sign of splenomegally, indicating a protective effect conferred by extracts against oxidative stress. The study, therefore suggests that these plants may play some key roles in alleviating salient nutritional, physiological and oxidative stress related challenges.

Keywords: ***Annona muricata*, *Fagara zanthoxyloide*, nutrients, bioactive components, oxidative stress, zidovudine**

INTRODUCTION

Several plants have been used by rural dwellers within Nigeria as a source of medicine and nutritional nourishments in periods of famine, drought, and civil unrest. With the increased interest in alternative medicines and healthy feeding which has been observed in the past decades, urban dwellers have widened their scope to embrace the possible nutritional and medicinal value attached to several plants observed around cities and towns as well as villages. The growing concern of the alternatives have spurred research into several plants to further broaden the genetic diversity and suggest varieties of nutritionally viable alternatives to the increasing medical and malnutrition problems observed in South-southern Nigeria (Adisa *et al.,* 2014). Of the vast array of plants which surround this region, two plants (*Annona muricata* and *Fagara zanthoxyloide*) have been used by 65% of traditional medicine dispensers to prepare medicinal concoctions for treatment and general body well being.

Annona muricata is one plant with widely acclaimed historical benefits to human beings and commonly referred to as sour soup in Nigeria. Its fruit is the main portion consumed by most people. This plant have been acclaimed to possess hepatoprotective, antihypertensive and antiplasmodic potentials; as well as been used in the treatment of neuralgia, palpitation, parturition, rashes, rheumatism, ringworm, spasms, tumors and ulcers with little mention of its nutritional potential. Notably, the leaves have been used traditionally in the treatment cystitis, diabetes, insomnia and headaches (Mishra *et al*., 2013), coughs, skin diseases and pains (Moghadamtousi *et al*., 2015). According to Abdul-Wahab *et al*., (2018), the roots have been acclaimed to have anti-inflammatory and anthelmintic potentials (Adewole and Ojewole, 2009). They leaves, fruits and roots have also been used as insecticides and pesticide agents among Africans as well as insect repellants (Abdul-Wahab *et al*., 2018).

Fagara zanthoxyloide is another ethnomedicinal plant which belongs to the family *Rutaceae*. It is an indigenous south-southern Nigeria plant that is widely used as a chewing stick for tooth cleaning in West Africa. *Fagara zanthoxyloide* has been acclaimed to possess antiplasmodial activity and used in the treatment of elephantiasis, impotence, sexually transmitted infections such as gonorrhoea, abdominal pain and malaria (Adefisoye *et al*., 2012).

Zidovudine, a potential inhibitor of HIV replication has been reported to increase mitochondrial lipid peroxidation and mitochondrial glutathione probably due to the releases of free radicals as well as reactive oxygen species in zidovudine treated animals (De la Asuncion et *al.,* 2004). Oxidative stress occur when the free radicals produced during normal cellular activities exceed the anti-oxidative capacity of the systems resulting in an increase in the radicals which leads to attack of the system's proteins, lipids and nucleic acids. The resulting cellular damage as a result of oxidative stress has been implicated to play a role in the pathogenesis of several diseases. In a bid to provide data to back up the belief displayed by traditional medicine dispensers on these plants, this study was carried out to investigate the nutrient, bioactive and effects of the extracts of *Annona muricata* leaves and *Fagara zanthoxyloide* roots on zidovudine induced oxidative stress in wistar rats.

MATERIALS AND METHOD

Collection of plant samples

The leaves and roots of *Annona muricata* were obtained from Alakahia community (3.92°N,7.80°E) in Obio/Akpor Local government Area of Rivers state; while leaves and roots of *Fagara zanthoxyloide* were obtained from Opoo community (8.28°N,3.67°E) in Itesiwaju Local Government Area of Oyo State. The plant materials were identified by Dr. Chikezie, .B. in the Department of Plant Science and Biotechnology, University of Port-Harcourt and the Chief Taxonomist, Dr. Olatunji, A., University of Ibadan Herbarium (UIH) with a voucher copy placed in the herbarium for reference.

Preparation of plant samples

The leaves (after separation from the stalk) and the roots were washed and air dried at room temperature (25˚C) until constant weight was obtained and milled with a Thomas-Willey (model 3383L40) mechanical grinder into a uniform coarse powder. The sample was stored in an air tight container until analysis.

Plant sample extraction

Three hundred grams of each of the dried powdered samples were placed in a conical flask and extraction was performed using 3 litres of absolute ethanol for a period of 1 week. The mixture was agitated at an interval of 48 hours on a rotary shaker. The extracts were centrifuged twice at 1500rpm for 15 min in a Wilten-bioteknika Microspin-12 LCM-3000 centrifuge, filtered with Whatman No 1 filter paper, evaporated to dryness at 40°C with a rotary evaporator and

lyophilized to recover the residue as sticky pastes which were stored at 4°C in a refrigerator for further to use.

The extracts of the leaves of *Annona muricata* and roots *Fagara zanthoxyloide* were used for animal studies based on the most common plant parts used by traditional medicine dispensers within this region.

Laboratory Animals

Acute toxicity study

The toxicity study was carried out using wistar albino rats (200-237g) divided into six groups of four rats each (one control group and 4 treatment group). They were acclimatized for seven days while on standard feed and water *ad libithum*. Treatment group were administered leaf extract of *Anonna muricata* and *Fagara zanthoxyloide* at 2,4,6,8 and 10 g/ml/Kg BW while the control group was administered only distilled water (2.5ml/kg orally). A graph of dose to experimental response was plotted for each extract from which the LD_{50} of the various extract was determined.

Experimental design

Healthy albino rats were purchased from the animal house of the Department of Biochemistry, University of Port Harcourt, Rivers state, Nigeria and divided into four groups (of 5 rats each) and housed in Griffin and George modular cage system.

Group 1 (Nor. Cntrl): Served as the normal control group which received no zidovudine or extract treatment

Group 2 (Neg. Cntrl): Served as the negative control group which received 100mg/ml of Zidovudine per Kg bw,

Group 3 (*AM*+ZDV): Served as *Annona muricata* treatment group which received 4.5g/ml/Kg bw of *Annona muricata* extract.

Group 4 (*FZ*+ZDV): Served as *Fagara zanthoxyloide* treatment group which received 3.8g/ml/Kg bw of *Fagara zanthoxyloide* extract.

All animals were treated in a manner that complied with the National Institute of Health (NIH) guidelines for the care and use of laboratory animals (NIH, 1985). Zidovudine was used to induce stress *in vivo* for two weeks in groups 2-4, prior to experimental treatment with extracts while treatment with extracts was performed for a period of 6 weeks.

METHODS

Proximate analysis

The proximate analysis would be performed by the method described in the Association of Official Analytical Chemists (AOAC, 2010).

Mineral analysis

The evaluation of the potassium, calcium, magnesium, copper, iron, sodium and potassium concentrations concentration according to procedures set aside in AOAC, 2010 and Achi *et al.*, (2017).

Vitamin analysis

The concentrations of retinol, α-tocopherol, thiamine, niacin, riboflavin, vitamin K and folate were assayed by the method stipulated by Achikanu *et al.*, (2013), AOAC, (2010) and Okwu and Josiah (2006).

Phytochemical Analysis

The concentrated extract samples were screened for phytochemical constituents according to methods described by Trease and Evans (2002) and Sofowora (2008).

Spectrophotometric quantification of the tannin, flavonoid, phenols, alkaloid and saponin content concentration in sample was performed by the method described by Ogunnka-Nnnoka *et al.,* (2019, 2004) and Ekwueme *et al.,* (2015).

Biomarkers of oxidative stress

Splenic homogenate was used for the assay of biomarkers of oxidative stress. Superoxide dismutase, catalase activity and reduced glutathione in spleen was determined by the method described by Fridovich (1989) and Aebi *et al*., (1983). Lipid peroxidation assay and splenic H_2O_2 concentration were performed by the methods of De Las Heras *et al*., (2003)

Histological analysis

The excised spleen was fixed with 10% formalin for 24hours, dehydrated in graded concentrations of xylene, embedded in molten paraffin wax and sectioned into 5 microns slices. The sectioned slices were fixed on glass slides and stained with haematoxylin and eosin (H&E) for examination with a microscope fitted with a camera unit. Photomicrography of sections of the tissues was taken and processed in a photo laboratory.

Statistical analysis

Results were expressed as Mean ± Standard error of mean with analysis of variance performed using SPSS software version 20 for Windows (SPSS Inc. USA) and Student t-test. The significant level during the test was set at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

The result of the proximate analysis of the roots and leaves of *Annona muricata* as well as *Fagara zanthoxyloide* (Table 1) showed that they contained high crude fibre, fat and protein. The leaves of *Annona muricata* was observed to possess high carbohydrate while the roots of Fagara zanthoxyloide possessed high ash content.

Table 1: Proximate composition of the leaves and roots of *Annona muricata* and *Fagara zanthoxyloide*

Proximate parameter	*Annona muricata*		*Fagara zanthoxyloide*	
	Leaves	Roots	Leaves	Roots
Crude protein (mg/g)	1.38 ± 0.03^{a}	$7.73 \pm 0.09^{a,c}$	$9.85 \pm 0.02^{c,d}$	10.54 ± 0.021^{b}
Crude fat (mg/g)	$9.73 \pm 0.11^{a,c}$	$6.37 \pm 0.04^{a,d}$	2.35 ± 0.015^{d}	$5.80 \pm 0.005^{b,c}$
Crude ash (mg/g)	0.65 ± 0.01^{c}	1.94 ± 0.04^{d}	$8.31 \pm 0.011^{a,b}$	9.47 ± 0.015^{c}
Crude fiber (mg/g)	$3.00 \pm 0.02^{b,c}$	8.27 ± 0.08^{c}	15.53 ± 0.005^{a}	$10.63 \pm 0.011^{a,d}$
Moisture (%)	18.30 ± 0.01^{b}	$13.38 \pm 0.18^{c,d}$	$10.32 \pm 0.011^{c,}$	$12.85 \pm 0.036^{c,d}$
Carbohydrate (%)	$65.23 \pm 0.12^{a,b}$	$52.76 \pm 0.33^{a,c}$	$50.19 \pm 0.011^{c,d}$	$55.32 \pm 0.011^{b,d}$

Values expressed as Mean ± SEM of triplicate determinations. values with same superscript are statistically significant ($p \leq 0.05$).

The high fibre present in the samples may aid digestion of food thus preventing constipation, result in a reduction of cholesterol levels in the serum (preventing cardiovascular-related disorders) and assist with the detoxification of carcinogens (Shemishere *et al.,* 2018). Crude protein present in samples may play a key role in transmission of neuro-informations and genetic traits, aid tissue repair and general body growth and well-being. The crude fat also observed to be present in all samples may indicate the possibility of samples to act as alternative energy sources.

The carbohydrate in *Annona muricata* leaves suggests that they may serve as good sources of energy with low susceptibility to spoilage due to the low moisture content inhibiting microbial

multiplication. The ash content of the roots of *Fagara zanthoxyloide* may suggest the possibility of an appreciable amount of minerals present in the sample (Shemishere *et al.*, 2018).

Mineral analysis revealed significantly high ($p \leq 0.05$) concentrations of iron, copper, zinc and calcium in the leaves of *Annona muricata* when compared with the roots which had a moderate concentration of phosphorous, calcium, zinc and sodium. The roots and leaves of *Fagara zanthoxyloide* were observed to be also rich in the following minerals iron, copper, zinc, and calcium.

Table 2: Mineral content of the leaves and roots of *Annona muricata* and *Fagara zanthoxyloide*

	Annona muricata		*Fagara zanthoxyloide*	
	Leaves	Roots	Leaves	Roots
Calcium (%)	$3.67 \pm 0.06^{a,c}$	$1.59 \pm 0.01^{c,d}$	0.19 ± 0.020^{a}	$1.03 \pm 0.015^{b,d}$
Magnesium (mg/100g)	$3.04 \pm 0.01^{b,d}$	$2.18 \pm 0.005^{b,d}$	$0.27 \pm 0.01^{c,d}$	$0.47 \pm 0.040^{a,c}$
Sodium (%)	$0.36 \pm 0.38^{a,d}$	1.08 ± 0.015^{a}	$0.27 \pm 0.350^{b,c}$	$0.167 \pm 0.011^{b,d}$
Potassium (%)	$0.47 \pm 0.021^{c,e}$	1.68 ± 0.040^{c}	$0.28 \pm 0.005^{a,e}$	$0.57 \pm 0.012^{c,d,e}$
Zinc(mg/100g)	0.34 ± 0.040^{c}	$1.35 \pm 0.010^{d,e}$	5.16 ± 0.02^{d}	$5.32 \pm 0.011^{d,e}$
Iron (mg/100g)	$20.23 \pm 0.01^{b,d}$	$5.21 \pm 0.02^{a,d,e}$	$10.01 \pm 0.01^{b,c,d}$	$15.02 \pm 0.02^{c,e}$
Copper (mg/kg)	2.17 ± 0.011^{a}	$0.16 \pm 0.01^{a,b}$	$2.53 \pm 0.011^{b,c}$	$7.38 \pm 0.017^{a,c}$

Values expressed as Mean ± SEM of triplicate determinants. values with same superscript are statistically significant ($p \leq 0.05$).

Plants with similar mineral constituents with that observed in *Annona muricata* and *Fagara zanthoxyloide* from this region have been known to control osmotic balance, reduce blood pressure as well as aid in bone formation. The presence of calcium in high amounts in the leaves of *Annona muricata* may be essential for blood clotting (hence coping with internal haemorrhage), bone formation, contraction of muscles, normal functioning of the respiratory and nervous systems as well as a vital co-factor for the process of erythropoiesis. Iron has been known to play a part in haemoglobin formation as well as aid in the oxidation of biomolecules (Thomas and Krishnakumari, 2015). In synergy with copper and cobalt, iron as observed in *Moringa oleifera* may stimulate bone marrow activity and enhance red blood cell production and maturation. Thus, their presence in these plants studied may help in the use of these plants as blood boosters. Copper on the one hand, also aid proper absorption of iron from the gastrointestinal tract, thereby increase iron concentration as well as boosting iron stores. The

minerals in these plants may thus be used to combat micronutrient deficiency. Zinc also observed in *Fagara zanthoxyloide* is known to play a pivotal role as essential components of several enzyme systems such as carbonic anhydrase, alanine peptidase, carboxypeptidase, carbonic anhydride. The impact of zinc is a salient one, but on the average about 20% of children in Nigeria is at risk of in adequate zinc intake with values of micronutrient deficiency in south-southern Nigeria increasing by the day (Dioxin and Harris, 2004). Thus, its presence in these plants may imply the benefit of the plants to protein synthesis, cell differentiation and replication as well as increased immunity as immune cells require iron, copper and zinc for their continuous generation in the bone marrow. These minerals are thus important for the normal functioning of all cells and numerous metabolic pathways.

Analysis of vitamins revealed varying concentrations of vitamins with significantly high ($p \leq 0.05$) concentrations of folate and ascorbic acid observed in both *Annona muricata* and *Fagara zanthoxyloide*. Leaves of *Annona muricata* were observed to contain significantly higher ($p \leq 0.05$) concentrations of vitamins D, E and K. The vitamin components of these plants may prove their relevance in several nutritional deficiency disorders.

Table 3: Vitamin content in the leaves and roots of *Annona muricata* and *Fagara zanthoxyloide*

	Annona muricata		*Fagara zanthoxyloide*	
	Leaves	Roots	Leaves	Roots
Rectinol (µg/100g)	$3.81\pm0.14^{a,c}$	$1.97\pm0.09^{a,c,d}$	0.16 ± 0.17^{b}	ND
Niacin (mg/Kg)	4.86 ± 0.19^{a}	$4.23\pm0.32^{c,d}$	$9.18\pm0.19^{d,e}$	$8.23\pm0.81^{d,e}$
Riboflavin (mg/kg)	$9.72\pm0.29^{c,e}$	$7.89\pm0.11^{a,d,e}$	$6.28\pm0.02^{c,e}$	$10.21\pm0.27^{c,d}$
Folate (mg/Kg)	$26.82\pm0.48^{a,b}$	$23.47\pm0.03^{b,c}$	$15.82\pm0.18^{a,c}$	$20.63\pm0.91^{,d,e}$
Ascorbic acid (mg/Kg)	$31.97\pm0.03^{a,b}$	$26.89\pm0.19^{d,e}$	$13.86\pm0.13^{c,e}$	$30.21\pm0.01^{c,d}$
Vitamin D (mg/Kg)	$4.21\pm0.21^{c,d,e}$	$0.91\pm0.16^{c,e}$	$1.11\pm0.26^{b,d}$	$3.21\pm0.49^{b,d,e}$
Vitamin E (mg/Kg)	$5.82\pm0.01^{a,d}$	$0.18\pm0.19^{c,d}$	$0.27\pm0.48^{a,c,e}$	5.08 ± 0.04^{c}

Values expressed as Mean ± SEM of triplicate determinants. values with same superscript are statistically significant ($p \leq 0.05$). *ND = Not detected

Ascorbic acid have been known to be an antioxidant which reduces the concentration of reactive oxygen species in the body and as result increases the immunity and decrease perioxidation (Gora *et al.,* 2006). Scurvy haemorrage a condition common to this region may be alleviated by

these plants due to the ascorbic acid content of which its deficiency results in weakening of the endothelial wall of the capillaries around the gums. The presence of vitamin E and riboflavin have also been known to induce antioxidant properties when consumed and this protects cells of the body against free radical-induced oxidative damage. The absence of these vitamins predisposes cell membrane to damages resulting in anaemia. A diet rich in riboflavin has also been linked to the proper maintenance of the connective tissues thus facilitating wound healing. Niacin and riboflavin also present in plants may aid co-enzyme formation leading to increased oxidative phosphorylation and thus energy production through the electron transport chain. Rectinol although observed in small quantities in the plants used in this study may in conjunction with ascorbic acid lead to an increase in the iron absorption from the gastrointestinal tract and its release from iron stores. This would thus promote the proliferation of the red blood cells in the bone marrow and reduce anaemic related condition observed among young women and geriatric individuals in this region.

Phytochemical analysis of the plants revealed the presence of alkaloids, flavonoid, tannins, phenols and steroids in the leaves and roots of both plants used in the study. Saponin was however not observed in the roots of *Annona muricata* but present in *Fagara zanthoxyloide* as as illustrated in Table 4. Quantitative phytochemical examination revealed significantly high ($p \leq 0.05$) concentrations of phytochemicals in the leaves of *Annona muricata* than in the roots with the exception of anthraquinone content which was observed to be significantly higher ($p \leq 0.05$) in the roots when compared with the leaves.

Table 4: Phytochemicals in leaves and roots of *Annona muricata* and *Fagara zanthoxyloide*

Phytochemical	*Annona muricata*		*Fagara zanthoxyloide*	
	Leaves	Roots	Leaves	Roots
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Terpernoids	+	+	+	+
Saponins	+	-	+	+
Phenols	+	-	+	+

Present: +, **Absent**: -

The result of quantitative phytochemical analysis on *Fagara zanthoxyloide* revealed a high concentration of phytochemicals in the roots on comparison with the leaves. The roots were observed to be rich in alkaloids, tannins and terpenoid which were also significantly higher ($p \leq 0.05$) in roots than in the leaves (Table. 5).

The presence of a significant array of phytochemicals in the leaves of *Annona muricata* and the roots of *Fagara zanthoxyloide* may be the reason for their preferred use by traditional medicine dispensers within this region than the other plant part investigated.

Table 5: Quantitative phytochemicals in the leaves and roots of *Annona muricata* and *Fagara zanthoxyloide*

Phytochemical (mg/100g)	*Annona muricata*		*Fagara zanthoxyloide*	
	Leaves	Roots	Leaves	Roots
Alkaloids	27.34 ± 0.15^{a}	$12.98 \pm 0.98^{a,b}$	$35.55 \pm 0.95^{a,c}$	$50.90 \pm 0.83^{b,c}$
Flavonoids	$19.66 \pm 0.04^{c,d,e}$	$3.71 \pm 0.46^{b,c}$	$3.27 \pm 0.34^{c,e}$	$8.63 \pm 0.27^{a,c}$
Tannins	$11.24 \pm 0.05^{a,c}$	$3.86 \pm 0.22^{c,d}$	$28.70 \pm 0.19^{a,e}$	$55.37 \pm 0.47^{b,c,e}$
Terpernoids	$8.19 \pm 0.11^{b,d}$	$5.21 \pm 0.19b,^{c}$	$18.23 \pm 0.08^{c,d,e}$	41.21 ± 0.16^{c}
Saponins	$6.32 \pm 0.14^{a,e}$	$1.25 \pm 0.07^{a,d,e}$	$7.43 \pm 0.41^{a,d}$	$19.44 \pm 0.59^{a,e}$
Phenols	$15.10 \pm 0.11^{a,c}$	$0.07 \pm 0.42^{b,c}$	$2.17 \pm 0.2^{a,d}$	$13.23 \pm 0.17^{c,d}$

Values expressed as Mean ± SEM of triplicate determinants. values with same superscript are statistically significant ($p \leq 0.05$).

The phytochemicals seen in the roots and leaves of *Annona muricata* and *Fagara zanthoxyloide* have been suggested in several studies to elicit several physiological properties. Notably, the high phenol content as seen in these plants may induce haematopoietic responses as well as confer antioxidant properties which have been implicated in treatment and management of haemolytic anaemia (Oboh and Akindahunsi, 2004). This possibly may be one of the reasons for the proposed use of the leaves of *Annona muricata* and the roots of *Fagara zanthoxyloide* by traditional medicine dispensers within this region for the management of symptoms of sickle-cell anaemia (Dubost *et al.,* 2007). Generally, the presence of terpenoid has been known to elicit stimulation of the immune system (Llauradó *et al*., 2012) as such these plant extracts may be applied in the management of secondary immunodeficiency conditions such as; HIV/AIDs, graft *vs* host diseases, leukaemia and lymphoma. Tannins present in these plants have been implicated in the treatment of inflamed tissues and have been used in cancer prevention. Alkaloids which offer repellant properties to plants against predators and parasites have been known to also be

resourceful in HIV infection as well as intestinal infections which accompanies AIDs. All of these may contribute synergistically to the use of these plants in herbal medications within this region.

Acute toxicity (LD_{50})

Result of the acute toxicity study showed on the leaf extract of *Annona muricata* and *Fagara zanthoxyloide* are illustrated in Tables 6 and 7. The results revealed that administration as from 8g/ml/Kg bw for *Annona muricata* and 6 g/ml/Kg bw for *Fagara zanthoxyloide* resulted in signs of toxicity and oral administration below this levels was well tolerated in mice even beyond 7 days. This result for the toxicity of *Annona muricata* was however slightly higher than the findings by Abdul-Wahab *et al.,* (2018) in which he observed kidney toxicity above 5g/Kg bw and Bertin *et al.,* (2017) who observed 100% mortality at 5g/Kg bw with *Annona muricata* from Benin was investigated.

Table 6: Acute toxicity test on *Annona muricata*

Group	Dosages (g/ml/Kg bw)	No. of rats used	No. of mortality	Remarks
Control	-	4	0	
1	2	4	0	
2	4	4	0	
3	6	4	0	
4	8	4	1	Salivation, weight loss.
5	10	4	2	Lacrimation, reduced irritability

Table 7: Acute toxicity test on *Fagara zanthoxyloide*

Group	Dosages (g/ml/Kg bw)	No. of rats used	No. of mortality	Remarks
Control	-	4	0	
1	2	4	0	
2	4	4	0	
3	6	4	1	Reduced irritability, fur coat changes observed
4	8	4	2	Muscle paralysis, weight loss.
5	10	4	2	Weakness and salivation

Fagara zanthoxyloide acute toxicity test result showed signs of toxicity as from 6g/Kg bw with animals witnessing weight loss, lacrimation and reduced irritability (Table 7). The median acute

toxicity value (LD_{50}) which was estimated at 3.8g/ml/Kg bw which suggests that the extract possessed no short time toxicity. This value was also similar with what was observed for *Fagara zanthoxyloide* root-peels by Ogwal-Okeng *et al.,* (2003) at 5g/ml/Kg bw however the reduced irritability at dosages higher than the LD_{50} may possibly culminate in the findings of Ogwal-Okeng *et al.,* (2003), suggesting a direct effect of extracts on nervous system.

The results of the *in vivo* antioxidant enzyme activities are illustrated in Table 8 below. The pre-administration of zidovudine resulted in an increase in superoxide dismutase (SOD) and catalase (CAT) activity at week 2 when compared to the normal control group. The Glutathione (GSH) concentration also followed a similar trend with a significant increase which continued as zidovudine administration progressed. Treatment with extracts of *Annona muricata* and *Fagara zanthoxyloide* resulted in a significant decrease ($p \leq 0.05$) in the SOD and CAT activity as well as GSH concentration which continued to decrease as time progressed. H_2O_2 and LPO results as seen in Table 9 showed a significant increase in the concentrations of these markers in the negative control group at week 2. Continuous administration with zidovudine resulted in further increases as seen at weeks 4 and 6. However the treatment with *Annona muricata* and *Fagara zanthoxyloide* extracts resulted in a significant decrease ($p \leq 0.05$) in the concentration of these markers in the tissue when compared to the negative control group. In the treatment group, the concentration of these markers decreased to approach that observed for the normal control group.

Table 8: Antioxidant potentials in zidovudine pre-administered albino rats treated with extract of *Annona muricata* leaves and *Fagara zanthoxyloide* roots.

	SOD (µmole of epinephrine oxidized/min/mg protein)			CAT (µmole of H_2O_2 consumed/min/mg protein)			GSH (µmole/mg protein)		
GRP	**Week 2**	**Week 4**	**Week 6**	**Week 2**	**Week 4**	**Week 6**	**Week 2**	**Week 4**	**Week 6**
Nor. Cntrl		4.25±0.11 [a]			15.50±0.06 [a,c,d]			20.67±0.09 [a,c]	
Neg. Cntrl	7.40±0.01 [b,d]	8.41±0.20 [b,d]	9.49±0.08 [b]	21.25±0.02 [a,c]	24.60±0.03 [a,b]	27.28±0.03 [a]	28.33±0.20 [a,c]	32.84±0.30 [b,d]	36.37±0.05 [b,c]
AM + ZDV	4.32±0.01 [a,b]	4.24±0.62 [b]	4.22 ±0.04 [a]	19.54±0.10 [a,d]	18.42±0.05 [b,d]	17.38±0.05 [a,c]	22.42±0.01 [b,c,d]	21.13±0.07 [a,b]	19.84±0.07 [c,d]
FZ + ZDV	4.86±0.20 [c,d]	3.50±0.03 [a,d]	3.27±0.20 [c,d]	18.96±0.27 [b,c]	16.78±0.81 [c,d]	14.81±0.08 [a,b]	23.61±0.36 [c,d]	19.70±0.18 [c]	19.41±0.17 [a,d]

Values expressed as Mean ± SEM of triplicate determinants. values with same superscript are statistically significant ($p \leq 0.05$).

Table 9: Oxidative stress makers in zidovudine pre-administered albino rats treated with extract of *Annona muricata* leaves and *Fagara zanthoxyloide* roots.

GRP	H_2O_2 (µmole/mg protein)			LPO (µmole/mg protein)		
	Week 2	**Week 4**	**Week 6**	**Week 2**	**Week 4**	**Week 6**
Nor. Cntrl		28.62±0.10 [a]			15.02±0.05 [a,c]	
Neg. Cntrl	44.80±0.04 [a]	54.34±0.40 [a,b]	61.76±0.06 [a,d]	23.510.20 [c,d]	28.50±0.02 [b,c]	32.41±0.30 [a,c]
AM + ZDV	28.48±0.02 [c,b]	28.17±0.09 [a,d]	27.83±0.17 [c,d]	18.04±0.01 [a,c]	17.82±0.04 [b]	17.74±0.04 [b,c]
FZ + ZDV	28.90±0.77 [a,c,d]	28.76±0.13 [b,d]	28.38±0.21 [d]	20.03±0.40 [b,d]	17.41±0.69 [a,c]	17.08±0.06 [c,d]

. Values expressed as Mean ± SEM of triplicate determinants. values with same superscript are statistically significant ($p \leq 0.05$).

The study by (Vijayameena *et al.,* 2013) suggested that the leaves of *Annona muricata* were observed to possess the enzymatic antioxidant superoxide dismutase but the glutathione and catalase, together with the non-enzymatic antioxidants for instance vitamin C and vitamin E observed from the result of this study signifies the impact of this plant in oxidative stress related scenarios. This further buttress the findings of this study as majority of the antioxidant enzymes in animals require the use of certain minerals elements adequately present in these plants. This explains the results of the antioxidant and oxidative stress relieving potentials of the extracts made from these plants as seen in Tables 8-9. Also, the physiological antioxidant activity may be keyed to the effects of several bioactive compounds present in plants. The antioxidant potential exhibited by phenols stern from the ease of substitution of the hydroxyl groups in their aromatic rings. The presence of phenols, vitamins D and E, riboflavin may also be a reason for the reduction in the concentration of glutathione and the activities of superoxide dismutase, catalase in the spleens of rats used in the study and the proposed antioxidant effects of these plants.

The antisickling and anaemic curative potential of these plants may also stern from its antioxidant capability, as these plants may prolong the life span human blood cellular components by binding to their membrane and preventing it from oxidative damage as is the case with anaemia (Koren *et al*., 2010).

The result of the splenic histopathology (Plates 1-10) showed a reduction in the pore size of the splenic sinusoids seen in the negative control group as well as atrophy with no case of splenomegaly. Treatment with extract of *Annona muricata* and also *Fagara zanthoxyloide* resulted in the preservation of the architecture of the spleen with normalization of sinusoid space and absence of athropy and necrosis as observed in the negative control

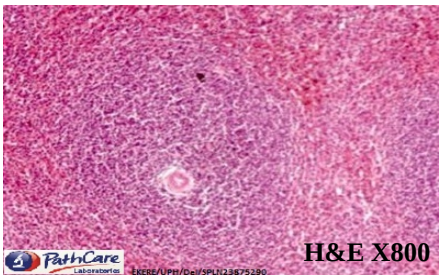

Plate 1. Spleen of normal control group showing evenly distributed red and white pulp regions with no pathological lesions.

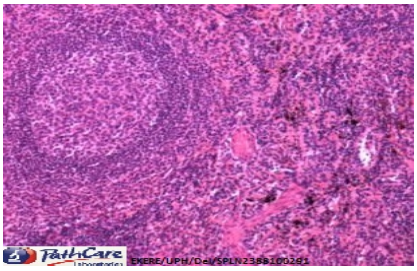

Plate 2. Spleen of Neg. control group at week 2 showing red and white pulp regions with parenchyma cells.

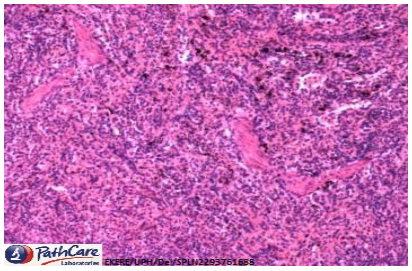

Plate 3. Spleen of Neg. control group at week 4 showing red and while pulp regions with infiltration of neutrophils and lymphocytes

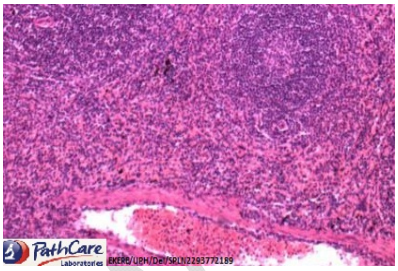

Plate 4. Spleen of Neg. control group at week 6 showing a normal central artery constrictions in red pulp with cyanocilic fibrosis and necrotic stroma.

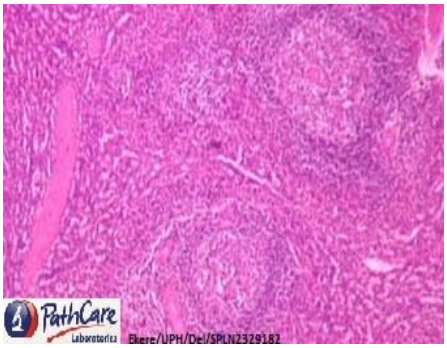

Plate 5. Spleen of *AM* + ZDV at week 2 showing white pulp with stream of myeloblast

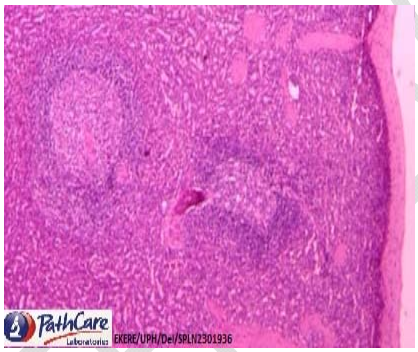

Plate 6. Spleen of *AM* + ZDV at week 4 showing splenic vacoulation and sinusoidal space.

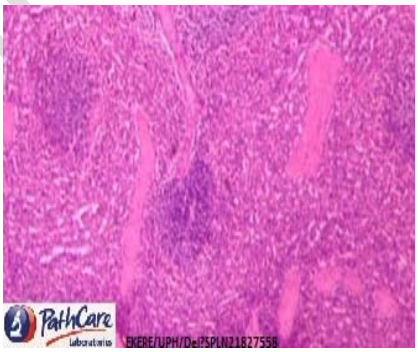

Plate 7. Spleen of *AM* + ZDV at week 6 showing lymphoid sheath and malpighian follicles

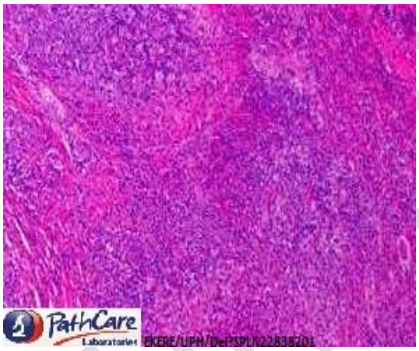

Plate 8. Spleen of *FZ* + ZDV at week 2 showing red blood sinusoids

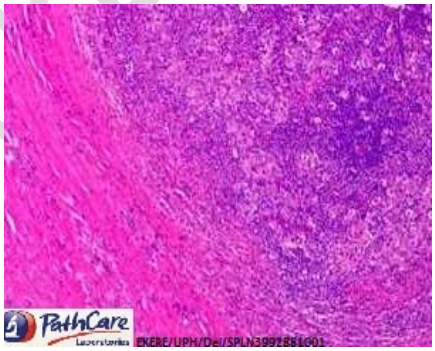

Plate 9. Spleen of *FZ* + ZDV at week 4 showing region of β-lymphocyte aggregation and lymph nodes

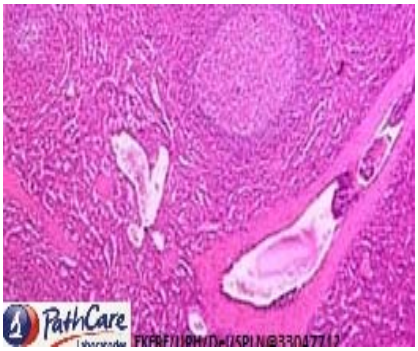

Plate 10. Spleen of *FZ* + ZDV at week 6 showing a lymphocyte around the splenic capsule

The results thus suggest that the extracts may confer protection against oxidative stress induced necrosis and athophy as observed in the negative control group, thus preserving the architecture of the white and red pulp enabling the spleen perform its quality control function on the haematopoietic system The restoration of sinusoidal pore sizes also buttress the protective effect

of the extracts on cell membrane of splenic sinusoid against membrane oxidation as proposed by (Chapman and Azevedo, 2018) which may be attributed to the prescience of phytochemical such as tannins, phenols and flavonoids as well as other mineral and vitamins which confer anti-oxidative properties to the extracts. Administration of extracts also resulted in increase in red blood cells and β-lymphocyte aggregation indicating normalization in the splenic functionality.

CONCLUSION

Plants are a great source of food and medicine for humans. The proposed acclaimed effect of these plants by traditional healers is due to the activity of several biochemical compounds in them. An analysis of *Annona muricata* and *Fagara zanthoxyloide* has revealed that these plants from this region accumulate a high amount of phytochemicals and possess vitamins and minerals which can help in cases of micronutrient deficiency as well as alleviate symptoms observed in several physiological conditions observed within this region. These bioactive components as seen from *in vivo* studies may also serve as potential antioxidants and aid in reducing oxidative stress derived from toxicants, heavy metals and free radicals present in the ecosystem.

REFERENCES

Abdul-Wahab, S. M., Jantan, I., Haque, M. A., Arshad, L. (2018). Exploring the leaves of *annona muricata* l. As a source of potential anti-inflammatory and anticancer agents. *Frontiers in pharmacology* **9**:661.

Achi, N.K., Onyeabo, .C., Ekeleme-Egedigwe, C.A, Onyeanula, J.C. (2017). Phytochemical, Proximate Analysis, Vitamin and Mineral Composition of Aqueous Extract of *Ficus capensis* leaves in South Eastern Nigeria. *Journal of Applied Pharmaceutical Science* **7**(03):117-122

Achikanu C.E, Eze-Steven .P.E, Ude C.M, Ugwuokolie O.C. (2013). Determination of the vitamin and mineral composition of common leafy vegetables in South Eastern Nigeria. *International Journal of Current Microbiology and Applied Sciences*, **2** (11): 347-353.

Adefisoye M.A, AjibadeAko-Nai, K., Bisi-Johnson, M.A. (2012). Phytochemical and antibacterial activity of the extracts of *Fagara zanthoxyloide*s on selected cariogenic and enteric bacterial isolates. *Journal of Intercultural Ethnopharmacology*. **1**(1):1-6

Adewole S., Ojewole J. (2009). Protective effects of *Annona muricata* Linn.(Annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of

streptozotocin-treated diabetic rats. *African Journal of traditional, complementary and Alternative medicine* **6**:30–41.

Adisa, O.I., Belonwu, D.C. , Oghenekaro, U.E ., Odeghe, O.B. (2014). Effect of selected yoruba medicinal formulations on hepatic architecture and functionality in wistar albino rats. *Global Journal of Research on Medicinal Plants & Indigenous Medicine* **3**(9):331–338

AOAC. (2010). Official Methods of Analysis. 18th Ed., Revision 3, Association of Official Analytical Chemists, Washington DC.

Belonwu, D. C., Onyieke, E. N., Ekere, O.U. (2013). Comparative phytochemistry of peddled yoruba medicinal formulations. *Indian Journal of Drugs and Diseases* **2**(3):259-270.

Belonwu, D. C., Onyieke, E. N., Ekere, O.U. (2013). Phytochemical Analysis of the Yoruba Medicinal Formulations- "Gbogbo Nise" and its Effect on Some Liver Enzymes. Indian Journal of Drugs and Diseases **2**(5):288-293.

Bertin, A.G., Euloge, S.A., Angelus, K., Edwige D., Alphonse S. and Sohounhloue D. (2017). Phytochemical and acute toxicity of ethanolic extract from leaves of Annona muricata (L.) from Benin in experimental albino rats. *International Journal of Chemical Studies* **5**(6): 39-41

Chapman, J. and Azevedo, A.M (2018). Splenomegaly. StatPearls Publishing, Treasure Island, Florida.

De la Asunción, J.G., Del Olmo, M.L., Gómez-Cambronero. L.G., Sastre, J., et al., (2004). AZT induces oxidative damage to cardiac mitochondria: protective effect of vitamins C and E. *Life sciences* 76(1):47-56

Dubost, .N., Ou, B. and Beelman, R. (2007). Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. *Food Chemistry*. 1**05**(2):727-735

Ekwueme, F.N., Nwodo, O.F., Joshua, P.E. *et al.* (2015). Qualitative and Quantitative Phytochemical Screening of the Aqueous Leaf Extract of Senna mimosoides : Its Effect in in vivo Leukocyte mobilization induced by inflammatory stimulus. *National Journal of Current Microbiology and Applied Sciences.* **4** (5): 1176-1188

Gora, D., Sandhya, M., Shiv, G. and Praveen, S. (2006). Oxidative stress, α-tocopherol, ascorbic acid and reduced glutathione status in Schzophrnics. *Indian Journal of Clinical Biochemistry*, 21: 34-38

Koren, .E., Kohen, .R., Ginsburg, .I. (2010). Polyphenols enhance total oxidant-scavenging capacities of human blood by binding to red blood cells. *Experimental Biology and Medicine.* **235**(6):689-99.

Llauradó, M., Abal, M., Castellví, J., Cabrera, S., Gil-Moreno, A., *et al*., (2012). ETV5 transcription factor is overexpressed in ovarian cancer and regulates cell adhesion in ovarian cancer cells. *International Journal of Cancer* **130**:1532–1543

Mishra S., Ahmad S., Kumar N., Sharma B. (2013). *Annona muricata* (the cancer killer): a review. *Global Journal of Pharmaceutical Research* **2:**1613–1618

Moghadamtousi S. Z., Fadaeinasab M., Nikzad S., Mohan G., Ali H. M., Kadir H. A. (2015). *Annona muricata* (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. *International Journal of molecular science* **16**:15625–15658**.**

Oboh, G. and Akindahunsi, A.A. (2004). Change in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. *Nutritional Health* **18**: 29-36.

Ogunka-Nnoka, C.U. and Mepba, H,D. (2008). Proximate composition and antinutrient contents of some common spices in Nigeria. *The Open Food Science Journal* **2**: 62-67

Ogunka-Nnoka, C.U., Ohwokevwo, O.A., Onyeike, E.N. (2019). Nutraceutical potentials of Splilanthes filicualis. *Global Journal of Science Frontier Research* **19**(1): 22-28.

Ogwal-Okeng, J. W., Obua, C., & Anokbonggo, W. W. (2003). Acute toxicity effects of the methanolic extract of Fagara zanthoxyloides (Lam.) root-bark. *African health sciences*, ***3***(3):124–126.

Okwu, D.E, and Josiah C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology*. **4**:357-361.

Shemishere, U.B., Taiwo, J.E., Erhunse, N. and Omoregie, E.S. (2018). Comparative Study on the Proximate Analysis and Nutritional Composition of *Musanga cercropioides* and *Maesobotyra barteri leaves. Journal of Applied and Science and Environmental Management* **22**(2):287 – 291.

Sofowora A. (2008). Medicinal Plants and Traditional Medicine in Africa. 3rd ed. Spectrum Books, Ibadan.

Thomas R.A and Krishnakumari S. (2015). Proximate analysis and mineral composition of *Myristica fragrans* seeds. *Journal of Pharmacognosy and Phytochemistry.* **3**(6): 39-42