# 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 of *Annona muricata* (*AM*) and the roots of *Fagara zanthoxyloide (FZ)* on zidovudine-induced oxidative stress in wistar albino rats. Wistar albino rats were divided into four groups of five rats each. Groups 2-4 were induced with 100g/ml/Kg bw of zidovudine (ZDV) 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 (0.65-9.45%), crude protein (1.38-10.54%), crude fat (2.35-9.73%), crude fibre (3.00-15.53%) and carbohydrate (50.19-65.23%). Iron was the highest mineral present in all the samples followed by zinc and calcium for *FZ* and *AM* respectively; while folate and ascorbic acid were the highest 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 ethanol extracts of the leaves of A*M* and roots of *FZ* reduced the activities of superoxide dismutase, catalase and glutathione. The splenic histology revealed atrophy, early onset necrosis and reduction in sinusoidal pore size in the negative control group which were absent in the extract treatment groups 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 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, towns and villages. The growing concern for 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 has been acclaimed to possess 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 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 medicated individuals (De la Asuncion et *al.*, 2004). Oxidative stress occurs 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. B. Chikezie in the Department of Plant Science and Biotechnology, University of Port-Harcourt and the Chief Taxonomist, Dr. A. Olatunji University of Ibadan Herbarium (UIH) with a voucher copy (UIH/034/8212) placed in the herbarium for reference.

### *Preparation of plant samples*

The leaves and the roots were sorted, washed with distilled water 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 (300g) 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 use.

***Laboratory Animals***

***Acute toxicity study***

The toxicity study was carried out using wistar albino rats (200g - 237g) divided into six groups with five rats each (one control group and 5 treatment groups) performed according to the Organization for Economic Cooperation and Development (OECD, 2011) as described in Ogbuehi *et al*., 2015. 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 (with 5 rats each) and housed in Griffin and George modular cage system. The extracts of the leaves of *Annona muricata* and roots *Fagara zanthoxyloide* were used for animal studies. 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.

**Group 1 (Normal control)**: received no zidovudine or extract treatment

**Group 2 (Negative control)**: 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* leaves extract.

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

**METHODS**

***Proximate analysis***

The proximate analysis was performed according to the method described by the Association of Official Analytical Chemists (AOAC, 2010).

***Mineral analysis***

The evaluation of the potassium, calcium, magnesium, copper, iron, sodium and potassium concentrations were performed according to the procedures by AOAC (2010) and Achi *et al.*, (2017).

***Vitamin analysis***

The concentrations of retinol, α-tocopherol, thiamine, niacin, riboflavin, vitamin K and folate were performed by the method of AOAC (2010) and Okwu and Josiah (2006).

***Phytochemical Analysis***

The concentrated extract samples were screened for phytochemical constituents according to the methods described by Sofowora (2008) and the quantitative constituents according to the method described by Ogunnka-Nnnoka *et al.,* (2019).

***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 were 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***

Histological examination of the excised spleen was performed by the method of Al-Hasawi and Al-Harbi (2014)

## STATISTICAL ANALYSIS

Results were expressed as Mean ± Standard error of mean with analysis of variance and Student t-test performed using SPSS software version 20 for Windows (SPSS Inc. USA). 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 (%) | $1.38 \pm 0.03^{a}$ | $7.73 \pm 0.09^{a,c}$ | $9.85 \pm 0.02^{c,d}$ | $10.54 \pm 0.021^{b}$ |
| Crude fat (%) | $9.73 \pm 0.11^{a,c}$ | $6.37 \pm 0.04^{a,d}$ | $2.35 \pm 0.015^{d}$ | $5.80 \pm 0.005^{b,c}$ |
| Crude ash (%) | $0.65 \pm 0.01^{c}$ | $1.94 \pm 0.04^{d}$ | $8.31 \pm 0.011^{a,b}$ | $9.47 \pm 0.015^{c}$ |
| Crude fiber (%) | $3.00 \pm 0.02^{b,c}$ | $8.27 \pm 0.08^{c}$ | $15.53 \pm 0.005^{a}$ | $10.63 \pm 0.011^{a,d}$ |
| Moisture (%) | $18.30 \pm 0.01^{b}$ | $13.38 \pm 0.18^{c,d}$ | $10.32 \pm 0.011^{c,}$ | $12.85 \pm 0.036^{c,d}$ |
| 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 content present in the roots and leaves of *FZ* may aid digestion of food thus preventing constipation. It also results in reduction of cholesterol levels in the serum (Shemishere *et al.,* 2018). The high crude protein observed in the roots and leaves of *Fagara zanthoxyloide* and roots of *Annona muricata* may play a key role in transmission of neuro-informations and genetic traits. 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 values in all the samples may suggest that they may serve as good sources of energy. The ash content of the roots and leaves of *Fagara zanthoxyloide* may suggest the possibility of an appreciable amount of minerals present in these samples (Shemishere *et al.,* 2018).

Mineral analysis revealed significant ($p \leq 0.05$) levels of iron in all the samples followed by zinc and calcium for FZ and AM respectively. Significantly ($p \leq 0.05$) high levels of magnesium and copper were recorded for AM leaves and roots of FZ respectively.

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

| | *Annona muricata* | *Fagara zanthoxyloide* |
|---|---|---|

| | Leaves | Roots | Leaves | Roots |
|---|---|---|---|---|
| Calcium (%) | $3.67 \pm 0.06^{a,c}$ | $1.59 \pm 0.01^{c,d}$ | $0.19 \pm 0.020^{a}$ | $1.03 \pm 0.015^{b,d}$ |
| 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 (%) | $0.36 \pm 0.38^{a,d}$ | $1.08 \pm 0.015^{a}$ | $0.27 \pm 0.350^{b,c}$ | $0.167 \pm 0.011^{b,d}$ |
| Potassium (%) | $0.47 \pm 0.021^{c,e}$ | $1.68 \pm 0.040^{c}$ | $0.28 \pm 0.005^{a,e}$ | $0.57 \pm 0.012^{c,d,e}$ |
| Zinc(mg/100g) | $0.34 \pm 0.040^{c}$ | $1.35 \pm 0.010^{d,e}$ | $5.16 \pm 0.02^{d}$ | $5.32 \pm 0.011^{d,e}$ |
| 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) | $2.17 \pm 0.011^{a}$ | $0.16 \pm 0.01^{a,b}$ | $2.53 \pm 0.011^{b,c}$ | $7.38 \pm 0.017^{a,c}$ |

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

The samples studied recorded appreciable amount of iron. Iron has been known to play a part in haemoglobin formation as well as aid in the oxidation of biomolecules. 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 suggest their usefulness in blood boosting. The high calcium content found in the leaves of *AM* 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. The high copper content in the roots of *FZ* shows that it can aid proper absorption of iron from the gastrointestinal tract, which leads to increase in iron concentration (boosting iron stores). Zinc also observed in the roots and leaves of *FZ* 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 inadequate zinc intake with values of micronutrient deficiency in south-southern Nigeria increasing by the day. 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. The minerals in these plants may thus be used to combat micronutrient deficiency.

Analysis of vitamins content revealed varying concentrations of vitamins with significantly high ($p \leq 0.05$) concentrations of folate and ascorbic acid observed in both *AM* and *FZ*. The leaves of

*Annona muricata* were also observed to contain significantly higher ($p \leq 0.05$) concentrations of vitamins D, E and K. The vitamin components of these plants may also 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) | $3.81 \pm 0.14^{a,c}$ | $1.97 \pm 0.09^{a,c,d}$ | $0.16 \pm 0.17^{b}$ | ND |
| Niacin (mg/Kg) | $4.86 \pm 0.19^{a}$ | $4.23 \pm 0.32^{c,d}$ | $9.18 \pm 0.19^{d,e}$ | $8.23 \pm 0.81^{d,e}$ |
| Riboflavin (mg/kg) | $9.72 \pm 0.29^{c,e}$ | $7.89 \pm 0.11^{a,d,e}$ | $6.28 \pm 0.02^{c,e}$ | $10.21 \pm 0.27^{c,d}$ |
| Folate (mg/Kg) | $26.82 \pm 0.48^{a,b}$ | $23.47 \pm 0.03^{b,c}$ | $15.82 \pm 0.18^{a,c}$ | $20.63 \pm 0.91^{,d,e}$ |
| Ascorbic acid (mg/Kg) | $31.97 \pm 0.03^{a,b}$ | $26.89 \pm 0.19^{d,e}$ | $13.86 \pm 0.13^{c,e}$ | $30.21 \pm 0.01^{c,d}$ |
| Vitamin D (mg/Kg) | $4.21 \pm 0.21^{c,d,e}$ | $0.91 \pm 0.16^{c,e}$ | $1.11 \pm 0.26^{b,d}$ | $3.21 \pm 0.49^{b,d,e}$ |
| Vitamin E (mg/Kg) | $5.82 \pm 0.01^{a,d}$ | $0.18 \pm 0.19^{c,d}$ | $0.27 \pm 0.48^{a,c,e}$ | $5.08 \pm 0.04^{c}$ |

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

Ascorbic acid present in significantly high ($p \leq 0.05$) concentrations in all samples has been known to be an antioxidant which reduces the concentration of reactive oxygen species in the body and as result increases 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 in the leaves of *AM* and roots of *FZ* have also been known to induce antioxidant properties when consumed thereby protecting cells of the body against free radical-induced oxidative damage. A diet rich in riboflavin as seen in all samples have also been linked to the proper maintenance of the connective tissues thus facilitating wound healing. Niacin and riboflavin common to both samples 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 leaves and roots of *AM* 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 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.

**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 leaves of *AM* and roots of FZ were observed to posses significantly high ($p \leq 0.05$) concentrations of all phytochemicals investigated when compared with the roots. 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 South-southern Nigeria 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 ± 0.98[a,b] | 35.55 ± 0.95[a,c] | 50.90 ± 0.83[b,c] |
| Flavonoids | 19.66 ± 0.04[c,d,e] | 3.71 ± 0.46[b,c] | 3.27 ± 0.34[c,e] | 8.63 ±0.27[a,c] |
| Tannins | 11.24 ± 0.05[a,c] | 3.86 ± 0.22[c,d] | 28.70 ± 0.19[a,e] | 55.37 ± 0.47[b,c,e] |
| Terpernoids | 8.19 ± 0.11[b,d] | 5.21 ± 0.19b,[c] | 18.23 ± 0.08[c,d,e] | 41.21 ± 0.16[c] |
| Saponins | 6.32 ± 0.14 [a,e] | 1.25 ± 0.07[a,d,e] | 7.43 ± 0.41[a,d] | 19.44 ± 0.59[a,e] |
| Phenols | 15.10±0.11[a,c] | 0.07±0.42[b,c] | 2.17±0.2[a,d] | 13.23±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. The high alkaloids content in all samples which offer repellant properties to plants against predators and parasites have been known to also be resourceful in intestinal infections which accompany immunodeficiency disorders. The tannin content significantly high in the leaves and roots of FZ has been implicated in the treatment of inflamed tissues. Generally, the presence of terpenoid as and leaves of *AM* 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. Notably, the high phenol content as seen in the leaves of *AM* and roots of *FZ* 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). 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 on the extract of *Annona muricata* leaves and *Fagara zanthoxyloide* roots are illustrated in Tables 6 and 7. The results revealed that administration as from 8g/ml/Kg bw for *Annona muricata* leaves and 6 g/ml/Kg bw for *Fagara zanthoxyloide*

roots resulted in signs of toxicity and oral administration below this levels was well tolerated in mice even beyond 7 days. The 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 extracts of *Annona muricata* leaves**

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

**Table 7: Acute toxicity test on extracts of *Fagara zanthoxyloide* roots**

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

*Fagara zanthoxyloide* acute toxicity test resulted in 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 suggests that the extract possessed no short time toxicity. This value was also similar with that 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.

Results on oxidative stress makers are shown in Figures 1-5 below. The CAT activity was observed to reduce in groups 3-4 when compared with group 2 with the values at week 6 observed to approach the activity observed in the normal control (Figure 2). GSH concentration was observed to decrease in groups 3 and 4 when compared with group 2 at week 2. The concentration in group 4 was also observed to decrease below the value observed for group 1 at weeks 4 and 6. No significant change was observed in the $H_2O_2$ concentration when the values in group 3 and 4 were compared with the normal control (Figure 4). The level of lipid peroxidation was observed to be significantly higher in groups 3 and 4 in comparison with the normal control but lower than that observed for the negative control (Figure 5), with the values in group 4 observed to significantly decrease at week 4 and 6. The administration of zidovudine resulted in an increase in superoxide dismutase (SOD) and catalase (CAT) activities, glutathione (GSH), hydrogen peroxide ($H_2O_2$) and lipid peroxidation (LPO) concentrations at week 2-6 (Figure 1-5). Treatment with extracts of *Annona muricata* and *Fagara zanthoxyloide* resulted in a significant decrease ($p \leq 0.05$) in these bio-markers which continued as time progressed. Treatment with *Fagara zanthoxyloide* roots caused a significant decrease ($p \leq 0.05$) in the SOD activity at weeks 4 and 6 when compared to all groups (Figure 1).

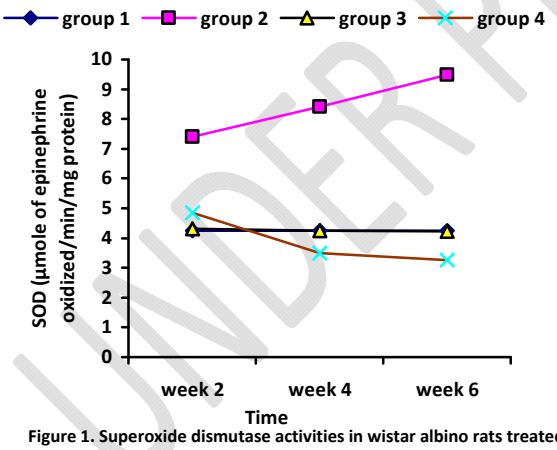



Figure 1. Superoxide dismutase activities in wistar albino rats treated with extracts of *Annona muricata* leaves and *Fagara zanthoxyloide* roots

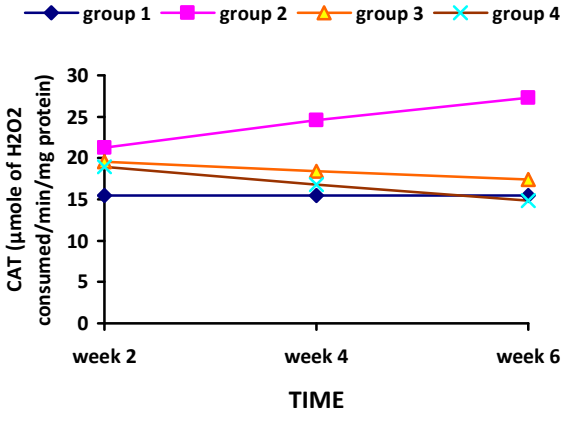



Figure 2. Catalase activities in wistar albino rats treated with extracts of *Annona muricata* leaves and *Fagara zanthoxyloide* roots

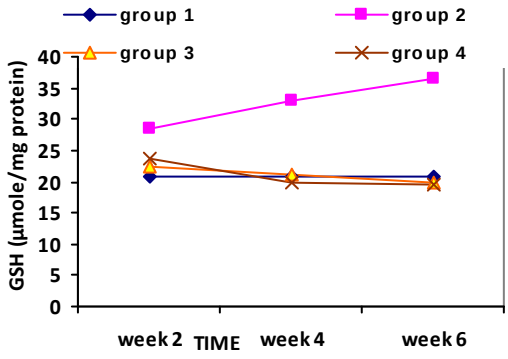



Figure 3. Gluthathione concentrations in wistar albino rats treated with extracts of *Annona muricata* leaves and *Fagara zanthoxyloide* roots

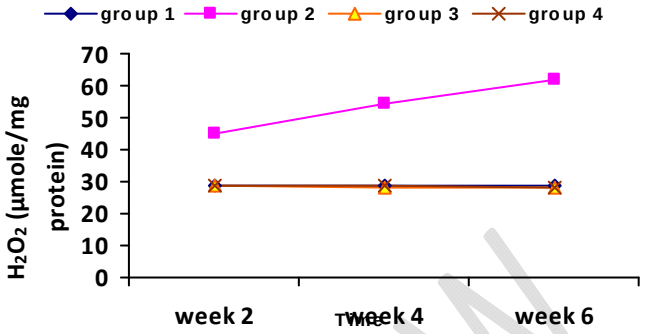



Figure 4. Hydrogen peroxide concentrations in wistar albino rats treated with extracts of *Annona muricata* leaves and *Fagara zanthoxyloide* roots

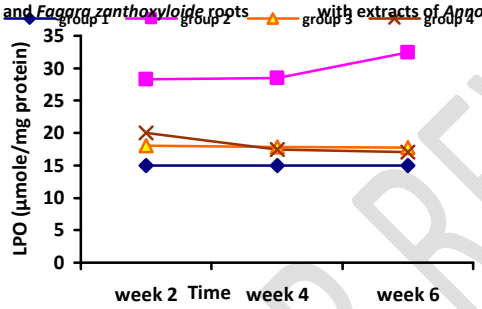


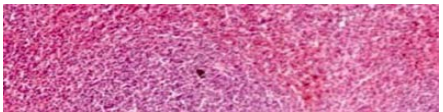

Figure 5. Lipid peroxidataion concentrations in wistar albino rats treated with extracts of *Annona muricata* leaves and *Fagara zanthoxyloide* roots

The reduction in the levels of these biomarkers on treatment with extract may suggest that the plants may serve as good sources of antioxidants which aid in alleviating cytotoxic effects of reactive oxygen species which results in damages to biological molecules, DNA, membrane function and ultimately ageing.

The reduction in the activities of the enzymatic antioxidants by the extracts of *Annona muricata* leaves and *Fagara zanthoxyloide* roots together with the presence of non-enzymatic antioxidants (phenols, riboflavin, ascorbic acid, vitamins D and E) observed in both plants may buttress the impact of these plants in oxidative stress related scenarios. These non-enzymatic antioxidants may serve as the reason for the reduction in the concentration of biomarkers of oxidative stress in the spleens of rats used in the study and the proposed antioxidant effects of these plants.

The result of the splenic histopathology showed a reduction in the pore size of the splenic sinusoids seen in the negative control group as well as atrophy and necrosis but no case of splenomegaly (Plate 2-4). 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 atrophy and necrosis as observed in the negative control. There





was however an observed increase in red blood cells and β-lymphocyte aggregation (Plate 9-10) in the group treated with *Fagara zanthoxyloide.*

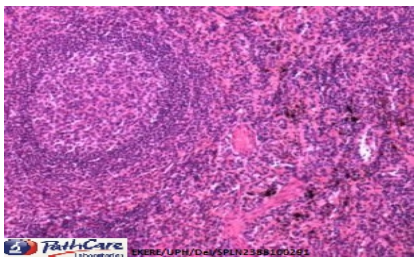


**Plate 2.** Spleen of group 2 at week 2 showing scared red and white pulp regions with parenchyma cells.

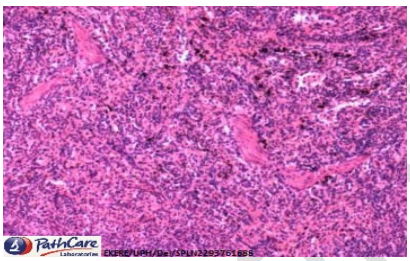


**Plate 3.** Spleen of group 2 at week 4 showing red and while pulp regions with infiltration of neutrophils and lymphocytes

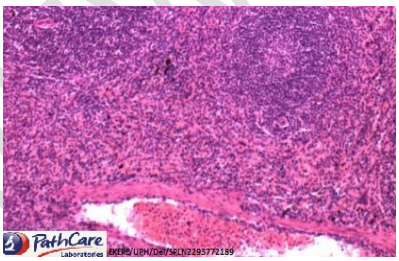


**Plate 4.** Spleen of group 2 at week 6 showing a normal central artery constrictions in red pulp with cyanocilic fibrosis and necrotic stroma.

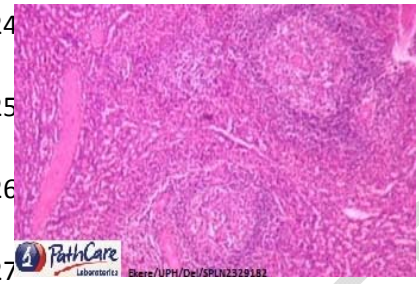


**Plate 5.** Spleen of group 3 at week 2 showing white pulp with stream of myeloblast

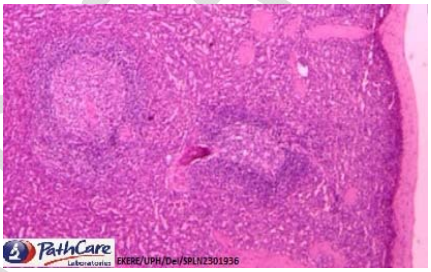


**Plate 6.** Spleen of group 3 at week 4 showing splenic vacoulation and sinusoidal space.

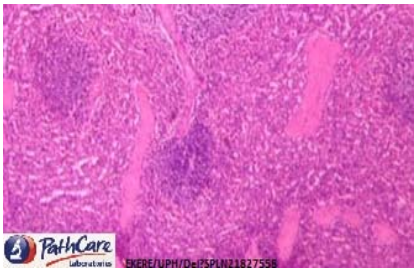


**Plate 7.** Spleen of group 3 at week 6 showing lymphoid sheath and malpighian follicles

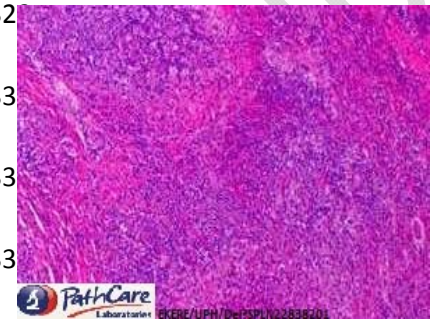


**Plate 8.** Spleen of group 4 at week 2 showing red blood sinusoids

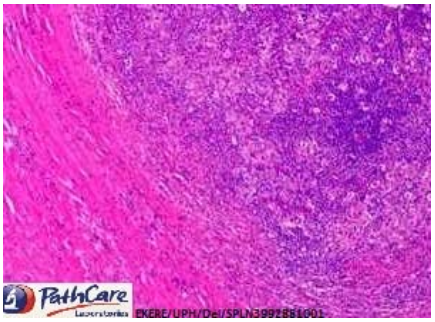


**Plate 9.** Spleen of group 4 at week 4 showing region of β-lymphocyte aggregation and lymph nodes

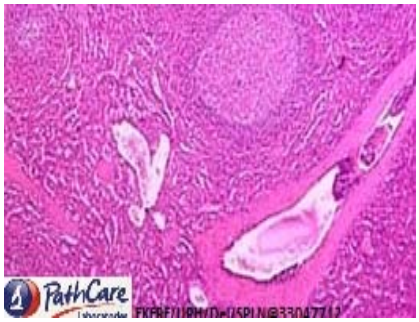


**Plate 10.** Spleen of group 4 at week 6 showing a lymphocyte around the splenic capsule

The results from the treatment groups (Plates 5-10) suggest that the extracts may confer protection against possibly oxidative stress induced necrosis and atrophy as observed in the negative control group (Plates 2-4), thus preserving the architecture of the white and red pulps and 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 membrane of splenic sinusoids against oxidation as proposed by Chapman and Azevedo, (2018) and may be attributed to the presence of phytochemicals such as tannins, phenols and flavonoids as well as other mineral and vitamins which confer anti-oxidative properties to the extracts. The increase in red blood cells and β-lymphocyte aggregation in the group 4 may indicate normalization in the splenic functionality on treatment with extracts of *Fagara zanthoxyloide* (Belonwu *et al.*, 2013a & 2013b).

## CONCLUSION

Plants are a great source of food and medicine for humans. The proposed acclaimed effect of *AM* and *FZ* by traditional healers may be due to the activity of several biochemical compounds in them. An analysis of *Annona muricata* and *Fagara zanthoxyloide* has revealed that these plants accumulate a high amount of phytochemicals and possess vitamins and minerals which can help in cases of micronutrient deficiency and alleviating symptoms observed in several physiological conditions. 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.

Ethical Approval:

As per international standard or university standard ethical approval has been collected and preserved by the authors.

## CONFLICT OF INTERESTS

The authors declare that they have no conflicting interests.

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