

Original Article**Free Radical Scavenging, Antimicrobial Activities and Effect of Sub-Acute Exposure to Nigerian *Xylopia aethiopica* Seed Extract on Liver and Kidney Functional Indices of Albino Rat**

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ABSTRACT

Background: *Xylopia aethiopica* is highly reputed for its numerous medicinal properties. In the present study, antioxidant, antimicrobial, and toxicity profile of methanol seed extract of Nigerian *X. aethiopica* in rat were evaluated.

Methods: Phytochemical compositions were evaluated using standard procedures. The antibacterial study was carried out using agar well diffusion method, while antioxidant activities were evaluated by DPPH and FRAP assay. Twenty-five rats (5 each) were given 0, 75, 150, 300 and 600 mg/kg bwt of the extract orally for 28 days.

Results: The extract had total phenolic and total flavonoid contents of 15.98±0.03mg GAE/g and 2.29±0.02 mg/g CE respectively. The extract had IC₅₀ values of 52.45±3.05 µg/mL and 73.45±3.89 µg/mL in DPPH and FRAP assay respectively. The *E. coli* showed the highest susceptibility (20.27±0.90mm) while *P. aeuruginisa* showed the least (15.08±0.20mm). The MIC ranged from 25-50 µg/mL while MBC ranged between 50µg/mL and 100 µg/mL. In comparison with the control rats, the levels of serum creatinine, bicarbonate total proteins, albumin, and ALP were significantly higher in rat dosed 600 mg/kg bwt while urea decreases in rat dose 300 and 600 mg/kg. However, serum concentration of ALT, AST, bilirubin, Na⁺, K⁺ and Cl⁻ compared favorably ($P>0.05$) with control at all doses.

Conclusion: The study revealed the antioxidant and antimicrobial activities of Nigerian *X. aethiopica*, the extract at 75, 150 and 300 mg/kg/b.wt did not provoke toxic effects to the animals' liver and kidney; however, caution should be exercised when using as a prolonged oral remedy at high doses.

Keywords: Antibacterial, Anti-Oxidants, Flavonoids *Xylopia aethiopica*, Phenolics, Toxicity.

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INTRODUCTIONS

Free radicals are reactive metabolite produce during redox reactions which in turn initiate chain of reactions that result in cellular damage [1]. There is considerable evidence implicating free radicals especially reactive oxygen species (ROS) in the etiology of more than one hundred degenerative disorders in humans [2]. Natural products contain active metabolites that protect against ROS [3].

For centuries, African medicinal plants/spices have been used in folk medicine for the treatment of different diseases and have been scientifically validated for their claimed activities [4-6]. Infectious diseases including bacterial infections continue to be a serious health concern worldwide [7]. About 50% of the world's populations are

susceptible to microbial infection [8]. About 17000000 out of 52000000 annual deaths, were attributed to microbial infections with young children having highest mortality rate (9000000) [8]. Meanwhile, conventional antibiotics are rapidly losing their effectiveness as bacteria and other microbes develop resistance to them [9]. This alarming increase in the development of resistance to the antibiotics in the clinical practice has led to the incidence of new and re-emergence of infectious diseases making them expensive and difficult to treat. Thus, research into new alternative for such microbes is warranted.

Spices are increasingly gaining scientific interest in the fields of nutraceuticals and functional foods, due to their potential health benefits. The increased

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search of medicinal value of spice is due to the therapeutic failure, unwanted adverse effect allied with synthetic drug and availability to the rural dwellers [10].

Xylopi aethiopica (Family: Annonaceae), commonly called Negro pepper, is an evergreen aromatic tree, that grows up to 20 m high. The seed of *X. aethiopica* is highly reputed for its numerous medicinal properties. In Africa, it is use as spice and local remedy for diarrhea, stomach ache, snakebite, cardiovascular diseases, diabetes, treatment and management of sexually transmitted infections in southern Nigeria [11]. It is used as an antiseptic to arrest bleedings after birth [12]. It has also been reported for inhibitory effect against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [13], and mild toxicity on *Clarias gariepinus* [14].

However, literature survey revealed scanty information on antioxidants, antimicrobial and safety evaluation of Nigerian indigenous *X. aethiopica*, the present study therefore was set up to fill the gap in knowledge.

MATERIALS AND METHODS

Xylopi Aethiopica

Fresh samples of *X. aethiopica* was obtained from the Lapai Market in Lapai, Niger State Nigeria and was identified by Botanist at the Department of Biological science Federal University of Technology, Minna.

Experimental Animals

Healthy albino rats (120.25±5.21) were procured from animals holding units of Federal University of Technology, Minna. They were allowed unrestricted access to rat pellets and water.

Chemicals and Reagents

Ascorbic acid (Merck Co.), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma-Aldrich Co.). All biochemical assay kits were either obtained from Randox Laboratories Ltd, United Kingdom or Agappe Diagnostics, Switzerland. All other chemicals were of analytical grade.

Preparation of Laboratory Sample

The *X. aethiopica* was washed and dried for 2 wk (37 °C) and finally grounded using a grinder mill. A 50 g of the plant material was extracted with 200mL of methanol using soxhlet apparatus and the resulting extract was concentrated using rotary evaporator.

Screening for Secondary Metabolites

The plant extract was analyzed for the presence of some secondary metabolite including alkaloids,

terpenes, tannins, saponins, phenols, steroids, phlobatannins and flavonoids using standard procedures [15].

Assay for Antibacterial Activity

P. aeuruginisa, *Salmonella typhi*, *Klebsiella pneumonia*, *S. aureus* and *Escherichia coli* were the isolates used for these experiments. Organisms were isolated using standard methods and maintained on agar slants and refrigerated for further use. Antibacterial activity of the extract was carried out using agar-well diffusion method [16]. A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract in triplicates [16].

Total Phenolic and Flavonoid Contents

Total phenolic content was analyzed using the Folin– Ciocalteu colorimetric method [17]. Results were expressed as mg gallic acid equivalent (GAE) per gram extract. Total flavonoid content was determined using the aluminum chloride colorimetric method [17]. The results expressed as mg quercetin equivalent (QE) per gram of extract. Each plant extract was prepared in triplicate

FRAP and DPPH Assay

DPPH radical scavenging activity of the plant extract at varying concentrations (2.5-100 µg/mL) was measured in vitro via the 2, 2'- diphenyl-1-picrylhydrazyl (DPPH) assay. While Fe³⁺ ion reducing power of the sample was evaluated using varying extract concentrations (2.5 - 100 µg/mL) according to the method of Oyaizu [18]. The extract concentration providing 50% inhibition (IC₅₀) was calculated from the plot of inhibition (%) against extract concentration. Ascorbic acid at the same concentrations was used as the reference antioxidants.

Toxicological Study

Acute toxicity was carried out as described previously [19]. In the subacute toxicity animals (5 each) were dosed 0 (control), 75, 150, 300 and 600 mg/kg bwt orally for 4 wk. Procedure [20] was followed during blood sample collection and serum preparation for biochemical analysis.

Biochemical Parameters

Serum activities of alkaline phosphatase (ALP), Aspartate transaminase (AST) and alanine transaminase (ALT) were determined as described previously [21]. The concentrations of serum total proteins, bilirubins, albumins, urea creatinine, sodium, potassium, and chloride were determine using standard methods [22-24].

Statistical Analysis

Values were analyzed using Statistical analysis system (SAS). Comparisons between different groups were carried out by analysis of variance, ANOVA ($P < 0.05$). Means differences were separated using Duncan's Multiple Range Test [25].

Ethical Approval

The principles governing the use of laboratory animals as laid out by the Federal University of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed.

RESULTS

Qualitative Phytochemicals, Total Phenol, and Flavonoid Contents

The phytochemicals compositions of *X. aethiopica* revealed the presence of phenols, tannins, alkaloids, saponins, terpenoids, flavonoids but absence of glycoside and anthraquinone (Table 1). Total phenolic and total flavonoid content of the extract was 15.98 ± 0.03 mg GAE per g and 2.29 ± 0.02 mg/g catechin equivalent respectively (Table 2).

Table 1. Phytochemical composition of methanol extract of *Xylopiya aethiopica*.

Phytochemicals	Negro pepper
Total Phenol	+
Total flavonoids	+
Tannins	+
Alkaloids	+
Saponins	+
Glycoside	-
Terpenoids	+
Anthraquinone	-

+ Means present and – means absent.

Table 2. Total phenol and flavonoid contents of methanol extract of *Xylopiya aethiopica*.

Phytochemicals	Contents
Total phenol	15.98 ± 0.03 mgGAE/g
Total flavonoids	2.29 ± 0.02 mg/g catechin equivalent

Values are mean \pm SEM of 3 determinations

Antioxidants Activities

Both methanol extract of *X. aethiopica* ($IC_{50} = 52.45 \pm 3.05$ $\mu\text{g/mL}$) and ascorbic acid (36.44 ± 1.78 $\mu\text{g/mL}$) promoted an inhibition of DPPH radical with increasing concentrations. (Fig. 1). The ability of extract to transform Fe^{3+} to Fe^{2+} as illustrated in

Fig. 2 shows that methanol extract of *X. aethiopica* had low % FRAP ($IC_{50} = 73.45 \pm 3.89$ $\mu\text{g/mL}$) compare to ascorbic acid ($IC_{50} = 24.39 \pm 0.46$ $\mu\text{g/mL}$).

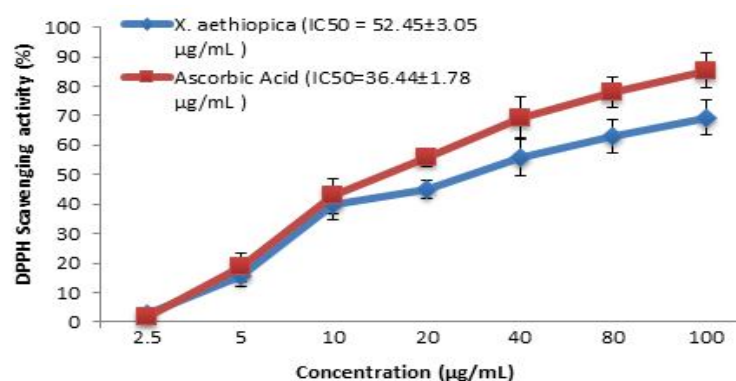


Figure 1. DPPH radical scavenging activities of methanol extract of *Xylopiya aethiopica*. Values are mean \pm SEM of 3 determinations. $IC_{50} = 52.45 \pm 3.05$ $\mu\text{g/mL}$ (*X. aethiopica*), 36.44 ± 1.78 $\mu\text{g/mL}$ (Ascorbic Acid).

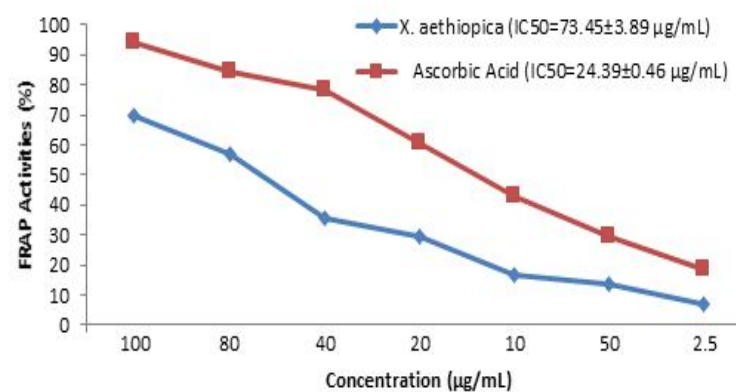


Figure 2. FRAP activity of leaf extracts of methanol extract of *Xylopiya aethiopica*. Values are mean \pm SEM of 3 determinations. $IC_{50} = 73.45 \pm 3.89$ $\mu\text{g/mL}$ (*X. aethiopica*), 24.39 ± 0.46 $\mu\text{g/mL}$ (Ascorbic Acid).

Antimicrobial Activities

The antibacterial activity assessed in terms of inhibition zone indicated that, at 12.5 $\mu\text{g/mL}$ and 25 $\mu\text{g/mL}$ of the extract, *P. aeuruginisa* and *Salmonella typhi* were not susceptible to the extract. However, at 50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ concentration of the extract, all organism tested were susceptible to the extract. *E. coli* showed the highest susceptibility of 20.27 ± 0.90 mm at 100 $\mu\text{g/mL}$ followed by *K. pneumoniae* 19.64 ± 0.54 mm, *S. typhi* (13.54 ± 0.23 mm) and *S. aureus* (12.67 ± 3.14 mm) while the least zone of inhibition of 15.08 ± 0.20 mm was recorded in *P. aeuruginisa* (Table 3). The MIC and MBC were 50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ respectively against *P. aeuruginisa* and *S. typhi*, while the MIC and MBC against *K. pneumoniae*, *S. aureus* and *E. coli* were 25 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$ respectively (table 4).

Table 3. Susceptibility of the test organisms to various concentrations of methanol extract of *Xylopi aethiopica*.

Test organism	Zone of Inhibition (mm)			
	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
<i>P. aeuruginisa</i>	-	-	5.86±0.23	11.78±0.68
<i>Klebsiella pneumoniae</i>	3.93±0.42	11.64±0.88	14.78±0.32	19.64±0.54
<i>Salmonella typhi</i>	-	-	7.84±0.24	13.54±0.23
<i>Staphylococcus aureus</i>	2.67±0.21	2.78±0.01	9.78±0.31	12.67±3.14
<i>Escherichia coli</i>	-	6.78±0.23	13.87±0.14	20.27±0.90
Control	7.74±0.03	13.14±0.82	19.24±0.28	25.24±0.29

Values are Mean ±SEM of triplicate determinations. Values with the same superscript alphabets are not significantly different ($P \leq 0.05$)

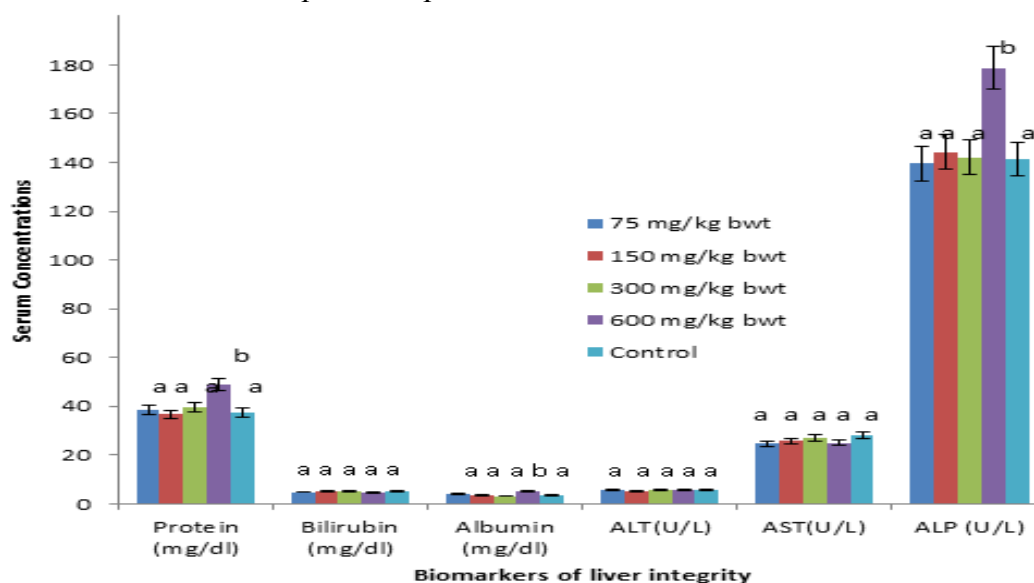
Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanol extract of *Xylopi aethiopica*.

Test organism	MIC µg/mL	MBC µg/mL
<i>P. aeuruginisa</i>	50	100
<i>Klebsiella pneumoniae</i>	25	50
<i>Salmonella typhi</i>	50	100
<i>Staphylococcus aureus</i>	25	50
<i>Escherichia coli</i>	25	50

Toxicological Study

Methanol extract of *X. aethiopica* when administered orally to rats had $LD_{50} > 5000$ mg/kg bw. The serum concentration of ALT, AST, and bilirubin in rats administered methanol extract of *X. aethiopica* compared favorably ($P > 0.05$) with control at all doses. The serum concentrations of total proteins, albumin, and alkaline phosphatase were significantly higher in rat dosed 600 mg/kg bwt of when compared with the control (Fig. 3). Serum concentration of sodium, potassium, and chloride in rats dosed 75, 150, 300 and 600 mg/kg b. wt of methanol extract of *X. aethiopica* compared

favorably ($P > 0.05$) with control at all doses. The serum concentration of creatinine and bicarbonate were significantly higher in rat dosed 600 mg/kg bwt of the extract. There were also significant decreases ($P < 0.05$) in concentrations of urea in rat dose 300 and 600 mg/kg of the extract when compared with the control rat (Fig. 4). The body weight gain and the computed liver, kidney, spleen, heart, and small intestine body weight ratios following 28 d administration of methanol extract of *X. aethiopica* (75, 150, 300 and 600 mg/kg b.wt) were not significantly ($P > 0.05$) different from those of their respective controls (Table 5).

**Figure 3.** Effect of subacute exposure to methanol seed extract of *X. aethiopica* on liver functional indices in rats.

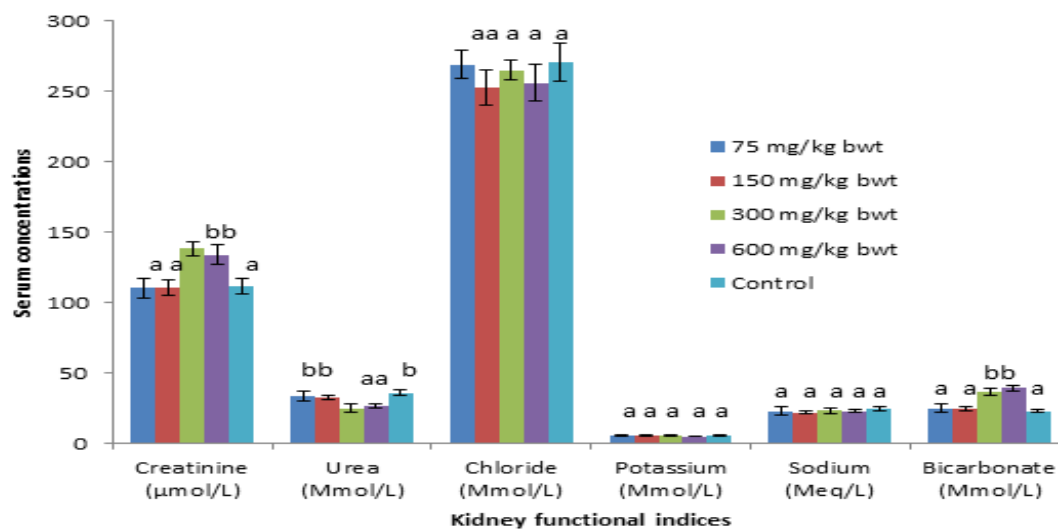


Figure 4. Effect of subacute exposure to methanol seed extract of *X. aethiopica* on kidney functional indices in rats.

Table 5. Body weight and relative organ weight ratio of rats administered methanol extract of *X. aethiopica*.

GROUPS	Liver	Heart	Kidney	Spleen	Initial weight (g)	body Initial weight (g)	body Weight gain(g)
75 mg/kg b.wt	2.38±0.18 ^a	0.64±0.00 ^a	0.95±0.02 ^a	0.39±0.12 ^a	132.00±1.45	157.73±2.34	25.73
150 mg/kg b.wt	2.39±0.22 ^a	0.63±0.01 ^a	0.96±0.01 ^a	0.42±0.21 ^a	124.32±1.54	151.65±3.46	27.33
300 mg/kg b.wt	2.13±0.20 ^a	0.61±0.02 ^a	0.92±0.01 ^a	0.44±0.09 ^a	128.89±1.67	158.23±3.95	29.34
500 mg/kg b.wt	2.59±0.12 ^a	0.62±0.01 ^a	0.89±0.00 ^a	0.39±0.44 ^a	132.86±1.78	162.23±5.34	29.37
CONTROL	2.24±0.10 ^a	0.63±0.00 ^a	0.93±0.00 ^a	0.37±0.01 ^a	124.32±2.34	155.08±4.08	30.76

Values are mean ± SEM of 5 determinations. Values along the same row with same superscripts are not significantly different ($P>0.05$).

DISCUSSION

Over the recent years, spices have gained greater attention as herbal medicines, alternative to conventional therapy. Phytochemical screening of *X. aethiopica* revealed the presence of phenols, tannins, alkaloids, saponins, terpenoids, flavonoids (Table 1). Various plant metabolites have been reported for their protective role against various diseases [26]. Among the great diversity of these secondary metabolites found in plants, flavonoids and phenol have achieved great scientific interest due to their role against pathogenic organism and in scavenging free radicals. The total phenolic (15.98±0.03 mg GAE/g) and total flavonoid (2.29±0.02 mg/g catechin equivalent respectively) contents of *X. aethiopica* would be chance of exploration of anti-microbial and antioxidant properties.

There is increasing interest in deleterious role of free radicals and preventive properties of natural products [4]. In the present study, methanol extract

of *X. aethiopica* promoted an inhibition of DPPH radical and the transformation of Fe^{3+} to Fe^{2+} in a dose-dependent fashion. The extract had IC_{50} values of 52.45±3.05 μg/mL and 73.45±3.89 μg/mL in DPPH and FRAP assay. The total phenolic and total flavonoid content of the extract recorded in this study could be responsible for the observed free radical scavenging activity of the plant extract.

Antimicrobial activity of methanol extract of *X. aethiopica* were evaluated with the aim of discovering novel bioactive agent against medically important pathogens, at concentrations of 12.5 μg/mL and 25 μg/mL there was no zone of inhibition on the *P. aeuruginisa* and *S. typhi* culture media thus signifying the absence of antimicrobial activity of the preparation against the *P. aeuruginisa* and *S. typhi*. However, at 50 μg/mL and 100 μg/mL concentration of the extract, favorable dose-dependent antagonistic activities against the representative gram-positive bacteria and gram-negative bacteria were exhibited by the extract. *E. coli* showed the highest susceptibility of

(20.27±0.90 mm) at 100 µg/mL followed by *K. pneumoniae* (19.64±0.54 mm), *S. typhi* (13.54±0.23 mm) while the least zone of inhibition of 15.08±0.20mm was recorded against *P. aeruginosa* (Table 3).

The observed dose-dependent antagonistic effect of the extract against the bacteria correlate with the argument [16], that increasing concentration of antimicrobial substance led to corresponding increase growth inhibition of pathogenic bacteria. Therefore, more of the antimicrobial agents were able to diffuse into the inoculated nutrient agar as the extract concentration increase

The antimicrobial activity of the crude extract is comparably lower than the activities recorded with standard antibiotics. These low activities may be due to the mixtures of bioactive compounds present in the extract compared to the pure compound contained in the standard antibiotics [27]. Nevertheless, at comparable dose of the active ingredient, unrefined crude extract rarely have the same degree of activity as the antibiotics [28]. Plant extract with MIC of <10 µg/ mL, < 100 µg/mL & > 100 µg/mL can be regarded as plant with significant, moderate and low antibacterial property respectively [29]. Based on this classification, methanol extract of *X. aethiopica* (MIC 25 - 50 µg/mL) exhibited moderate antagonistic effect against the bacteria tested.

In the acute toxicity, the LD50 was > 5000 mg/kg; this gram equivalence of the LD50 in an average adult man would translate to 350 gr dose of the drug. This is a very high value and makes the plant relatively safe for use. Evaluation of serum biochemical parameters including enzymes (aspartate transaminase, alanine transaminase, and alkaline phosphatase) and metabolites (total proteins, albumin, and bilirubin) are very useful in assessing the functional integrity of liver during subacute exposure of chemical substances or natural products/plant extracts [30].

The transaminase (ALT and AST) are enzymes of carbohydrate and amino acid metabolism while alkaline phosphatase is involved in hydrolysis of phosphate bonds. They are often used in assessing the functional integrity of liver, plasma membrane and endoplasmic reticulum [31].

Since the levels of ALT, AST, total proteins, albumin, sodium, creatinine and bicarbonate potassium and chloride and alkaline phosphatase bilirubin in rats dosed 75, 150 and 300 mg/kg b.wt of methanol extract of *X. aethiopica* showed no appreciable ($P>0.05$) increase or decrease with

respect to the control it implied that *X. aethiopica* had no harmful effect on the liver.

However, the significant increase in serum concentrations of total proteins, albumin and alkaline phosphatase in rat dosed 600 mg/kg b.wt of when compared with the control (Fig. 3), the functional integrity of liver has been compromised. The increase in the activity of ALP could be a consequence of activation of the enzyme or an increase in the rate of the synthesis of the enzyme induced by the higher dose of components of the extract [32]. The observed increase in the total protein and albumin content suggests a compromise of the synthetic ability of the liver arising from the administration of the extract. The extract might have increased the functional activity of the liver by interfering with the equilibrium in the rate of synthesis and destruction, removal or clearance of total protein and albumin from the system of the animals [33]. Such increase in total protein could, however, lead to dehydration which is detrimental to cellular homeostasis [32]. This will negatively affect the metabolic activities of the liver and consequently the health of the animals.

The kidneys control the excretion of urea, creatinine, and reabsorption of electrolytes into the blood. Defeat in activities of kidney will result in accumulation of electrolytes, urea, and creatinine in the biological fluid [34]. The absence of an effect on the levels of sodium, potassium, and chloride in rats dosed 75, 150, 300 and 600 mg/kg b.wt of methanol extract of *X. aethiopica* suggests that the normal excretion of this electrolyte by the kidney was not been adversely affected by the extract. The decrease in serum urea in rat dosed 300 and 600 mg/kg may be due to decreased protein catabolism or renal dysfunction [32], while the significant increase in creatinine content of the serum may be attributed to compromise of the renal functional capacity. The extract must have altered creatinine metabolism in favor of increase anabolism, decrease catabolism and decrease clearance [34].

However, administration of the *X. aethiopica* does not affect the body weight gain as well as the computed organs/body weight ratio of the animals thus suggesting that the extract did not cause any form of atrophy swelling, and hypertrophy on the organs [35].

CONCLUSION

The study revealed the antioxidant and antimicrobial activities of Nigerian *X. aethiopica*, the plant extract at 75, 150 and 300 mg/kg b.wt did not provoke toxic effects to the animals' liver and

kidney but could have some deleterious effects on kidney at 600 mg/kg bwt. Thus, caution should be exercised when using as an oral remedy for long

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