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Identification and Toxicity Profiling of Column Fractions of Ethanol Leaf Extract of *Ziziphus mauritiana*

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Abstract

Different plants may contain varying amounts of phytochemicals and also different degrees of toxicity. This study aimed to evaluate the phytochemicals present in the most active column fraction of ethanol leaf extract of the *Ziziphus mauritiana* plant and their toxic effects using brine shrimp lethality assay and animal model. Crude ethanol extract was obtained by maceration while fraction was achieved using a column chromatography experiment. Toxicity was evaluated with brine shrimp lethality assay and albino rat's models while characterization was achieved with liquid chromatography-mass spectrophotometry. *Ziziphus mauritiana* leaves revealed 42 fractions pooled into seven fractions. Fraction three (3) was the most toxic with the brine shrimp lethality assay of (31.48ug/ml) and its toxicological evaluation revealed an adverse effect on the hematological parameter, biochemical indices, and histo-architecture of the liver and kidney of the experimental model studied. LCMS analysis of the most toxic fraction revealed the presence of Antirrhinoside, Lucidumol A, Apigenin 7-glucuronide-4'-(6"-malonylglucoside), Dioscoreside C, Camellioside D, and others which have been reported for various pharmacological effects including adverse effects, The mode of toxicity may be synergistic, individual, or antagonistic which may explain the moderate toxicity observed in animal model. Administration of these fractions may lead to toxicity despite their potential.

Keywords: Dioscoreside C; chromatographic fractions; *Ziziphus mauritiana*; toxicity.

INTRODUCTION

It is important to bear in mind that different plants contain varying amounts of phytochemicals and toxicity levels. *Ziziphus mauritiana*, a perennial shrub or tree that can grow up to 3-15 meters tall with a trunk diameter of approximately 40 centimeters, has a variety of medicinal uses in folk medicine. Its leaves, fruits, and bark are used to treat a range of ailments, including diarrhea, dysentery, tuberculosis, and asthma (Kokwaro, 2009; Umair *et al.*, 2019). Additionally, certain parts of this plant have been found to possess beneficial bioactivities such as antioxidant, anticancer, and antidiarrheal activities (Abalaka et al., 2011; Jain *et al.*, 2019; Akanda and Hassan 2021; Dahiru *et al.*, 2006). However, there is a lack of information regarding the specific bioactive compounds present in these extracts and their potential effects. The aim of this study is to identify these compounds and evaluate any potential toxicological effects of the active fraction of the Ethanol extract of *Ziziphus mauritiana* leaves.

MATERIALS AND METHODS

Plant material Authentication

Fresh leaves of *Z. mauritiana* were obtained from Nigeria Police Academy, Wudil, Kano. Identification was carried out the Herbarium in the Department of Plant Biology, Bayero University Kano, Nigeria. Voucher number BUKHAN 0233 was allocated and the plant specimen were deposited.

Plant material preparation and Extraction

Fresh leaves of *Z. mauritiana* were dried in a ventilating room for Five (5) days and then ground into powdered form with the aid of an electric blender. 350g of the powdered leaves extracted by cold maceration method. The plant sample was soaked in 2000 ml of ethanol solution 99% and left to stand for 48 Hours. About 1700 ml of the filtrate were obtained and concentrated using a rotatory evaporator.

Column Chromatography and Structural Elucidation.

The column chromatography of the ethanol extract was carried out according to the method of Ode *et al.*, 2011 with modifications to suit this present study. Briefly, crude ethanol extract of *Ziziphus mauritiana* leaves weighing 20 grams was subjected to column chromatography in three (3) phases to separate the extract into its component fractions. Silica gel 60 by 120G was used as the stationary phase while varying solvent combinations (chloroform, Ethylacetate, ethanol, methanol, and water) of increasing polarity were used as the mobile phase. The following ratios of solvent combination was sequentially used in the elution process;

Hexane: Chloroform	100:0, 80:20, 60:40, 40: 60, and 20: 80;
Chloroform: Ethylacetate	100:0, 80:20, 60:40, 40: 60, and 20: 80;
Ethylacetate: Ethanol	100:0, 80:20, 60:40, 40: 60, and 20: 80;
Ethanol: Methanol	100:0, 80:20, 60:40, 40: 60, and 20: 80;
Methanol: Distilled Water	100:0, 80:20, 60:40, 40: 60, 20: 80 and 0:100.

A measured volume of 100ml of each solvent combination was collected and gradually poured uniformly by the sides of the glass into the column each time. This measure prevented solvent droplets from falling directly and disturbing the topmost layer of the column. Distortion of this layer would result in a non-uniform drain of the fractions. The eluted fractions was collected in aliquots of 50 ml in a glass container.

Analytical thin layer chromatography (TLC) and pooling of fractions

The content of each fraction was allowed to evaporate for 24-48 hours at room temperature with well-covered with perforated aluminum foil to prevent contamination. All fractions were spotted on pre-coated (silica gel F254) aluminum plates in a small chromatographic tank to pool them into different sub-fractions based on their relative mobility in solvent systems and colour reactions with an ultraviolet light lamp at 365 and 254 nm to identify the fluorescing spot and pattern of separation in the chromatogram. This procedure was used to pool the fractions and they were concentrated in a water bath to dryness. The mass of the different pooled fractions was determined and was kept at 40C in the refrigerator for further toxicological analysis.

Preparative thin layer chromatography (TLC) of most toxic pooled fraction

The most toxic pooled fraction was fractionated using preparative thin-layer chromatography. The preparative thin-layer chromatography method was carried out according to the method described by Ode *et al.*, 2011.

Structural Characterization of Most Toxic Fraction using Liquid Chromatography – Mass Spectrophotometery (LC-MS).

The phytochemical content of the most active sub-fraction was elucidated using liquid chromatography-mass spectrophotometry analysis at the Liquid Chromatography–Mass Spectrometry Laboratory at Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Selangor Darul Ehsan, Malaysia. The subfraction was diluted in methanol at a ratio of 1ml:1ml and injected into the LCMS system (Agilent 1290 Infinity LC system coupled to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source) with the following column (Agilent Zorbax Eclipse XDB-C18, Narrow-Bore 2.1x150mm, 3.5 micron (P/N: 930990-902)) conditions.

RESULTS AND DISCUSSION

RESULT

Column Chromatography yield of Ethanol leaf extract of *Ziziphus mauritiana.*

The result of chromatographic fractionation of the ethanol extract of *Ziziphus mauritiana* leaf is shown in Table 1. A total of seven (7) fractions were pooled from 41 preliminary fractions of ethanol crude extract of *Z. mauritiana* as shown in Table 1. Fraction one (1) yielded 5.3 grams, fraction two (2) 3.1 grams, and a total of 3.2 grams was obtained for fraction three (3). Fractions four (4), Five (5), six (6), and seven (7) yielded 3.9 grams, 1.8 grams, 1.9 grams and 1.4 grams respectively.

Table 1. Characteristic yield of fractions from the ethanol leave extract of *Ziziphus mauritiana.*

s/n	Fraction (s)	Weight obtained (Grams)
1	Fraction I	5.3
2	Fraction II	3.1
3	Fraction III	3.2
4	Fraction IV	3.9
5	Fraction V	1.8
6	Fraction VI	1.9
7	Fraction VII	1.4
	Total	20.6

Brine Shrimp Lethality Assay of fractions of ethanol leaf extracts of *Z. mauritiana*

The brine shrimp lethality assay of fractions of ethanol extract of Z. *mauritiana* leaf shown in Table 2 revealed that fraction three (3) gave the lowest lethal

concentration of 31.48 µg/ml followed by fraction four (4) and fraction one (1) with lethal concentration of 61.66µg/ml and 91.20 µg/ml respectively. Fractions 2, 5, 6, and 7 exhibit 123.47 µg/ml, 291.20 µg/ml, 261.60 µg/ml, and 231.47 µg/ml respectively.

Table 2. Brine Shrimp Toxicity of ethanol leaf extracts fractions of *Z. mauritiana.*

Extract	Dose (µg/ml)	Percentage Mortality	LC_{50} (µg/ml)	Toxicity Classification
Fraction I	0	0	91.20	Toxic /moderately toxic
	10	16.67		
	100	30.00		
	1000	93.33		
Fraction II	0	0	123.47	Slightly Toxic /Non toxic
	10	13.00		
	100	32.00		
	1000	52.45		
Fraction III	0	0	31.48	Toxic /moderately toxic
	10	33.00		
	100	63.00		
	1000	96.67		
Fraction IV	0	0	61.66	Toxic /moderately toxic
	10	30		
	100	56.67		
	1000	86.67		
Fraction V	0	0	291.20	Slightly Toxic /Non toxic
	10	20.00		
	100	50.00		
	1000	80.00		
Fraction VI	0	0	261.60	Slightly Toxic /Non toxic
	10	20.00		
	100	40.00		
	1000	80.00		
Fraction VII	0	0	231.47	Slightly Toxic /Non toxic
	10	10.00		
	100	30.00		
	1000	60.00		

Effect of Subchronic administration of fraction 3 (III) of Ethanol leaf extract of Ziziphus *mauritiana* on haematological Parameters.

The effect of fraction three (3) of ethanol leaf extract of *Ziziphus mauritiana* as shown in Table 3 revealed no significant difference in heamoglobin concentration in all groups treated with fraction 3 when compared with the control group. The red blood cell (RBC) concentration also showed no significant difference ($P<0.05$) among the groups (control, 200mg/kg and 500mg/kg groups).

The parked cell volume (PCV) values show no significant difference between the control group and group 2 administered with 200mg/kg while there was a decrease in the PCV concentration in group 3 (500mg/kg) which is statistically significant ($P<0.05$) when compared with the control group.

Mean Corpuscular Heamoglobin (MCH) shows there was no significant difference among the groups administered with different concentrations of fraction 3 of ethanol leaf extract of *Z. mauritiana* while Mean Corpuscular Heamoglobin Concentration (MCHC) also shows no significant difference when compared with the control. Mean Corpuscular Volume (MCV) shows there was no statistical difference between groups administered with 200mg/kg of the extract while a decrease was observed in the group treated with 400mg/kg when compared with the control group at $P<0.05$.

The WBC concentration showed a significant difference ($P < 0.05$) in all treated groups when compared with the control group, while Lymphocyte concentration and platelets also showed a decrease in concentration when compared with the control. MID concentration (average of monocytes, eosinophils, basophils) and neutrophils concentration showed no significant difference in all groups when compared with the control group.

Effect of Subchronic Administration of Fraction III (3) of Ethanol leaf extract of *Ziziphus mauritiana on* Liver Function Indices.

The effect of fraction III of ethanol leaf extract of *Ziziphus mauritiana* on liver function parameters is presented in Figures 1 and 2. It shows that there was a

significant increase in group 2 administered with 200mg/kg and group 3 administered with 500mg/kg when compared with the control for Aspartate aminotransferase (AST) concentration in the serum, while there was a significant difference in the concentration of Alanine aminotransferase (ALT) in group 3 (500mg/kg) only when compared the treated groups with the control group. The alkaline phosphatase (ALP) concentration shows no significant difference in all the groups treated with fraction III of ethanol leaf extract of *Z. mauritiana* when compared with the control group while there was no significant difference in MDA concentration between the control group and group 2 (200mg/kg) except group 3 (500mg/kg) (Figure 2) which exhibit a significant increase in the MDA concentration. Serum total protein reveals a significant decrease in group 3 (500mg/kg) while this decrease was not significant in group 2 (200mg/kg) when compared with the control, Albumin and Globulin reveal a decrease in all treated groups when compared with control group but were not significant($P<0.05$).

Effect of Subchronic administration of Fraction III (3) of Ethanol leaf extract of *Ziziphus mauritiana* on Kidney Function Indices

The effect of fraction 3 of Ethanol leaf extract of *Ziziphus mauritiana* on kidney function parameters as presented in Table 4 shows that there was no significant difference in creatinine, urea, sodium, and calcium concentrations between the control group and all the treated groups while potassium serum concentrations revealed an increase in group 3 administered with 500mg/kg body weight, for serum chloride ion concentration, a significant difference between the administered groups (200mg/kg and 500mg/kg) and the control group with decrease observed in groups 2 and 3.

Organ body-weight ratio Analysis of samples after Subchronic administration of fraction III of ethanol leaf extract of *Ziziphus mauritiana:*

The organ-body weight ratio of liver and kidney in Wistar rats administered with fraction III (3) as shown in Figure 3, the result shows a significant increase in the organs when compared with the control. The liver and kidney revealed an increase in weight with an increase in the dose administered and are significantly different from the control.

Histopathological Analysis of Liver and Kidney after Subchronic administration of fraction III of ethanol leaf extract of *Ziziphus mauritiana*

The Histopathological evaluation of the liver cell architecture shows unremarkable liver tissue for plate (control) with all the portal triad in position while plate (B) (200mg/kg) Shows areas of necrosis and inflammation (arrow point at areas of necrosis and inflammation) of liver tissue structure. Plate(C) (500mg/kg) also reveals areas of necrosis and inflammation.

The kidney cell architecture revealed no significant pathology and the cellular arrangement is intact for plate (A) (control) while plate (B)(200mg/kg) Shows areas of inflammation. Plate (C) (500mg/kg) reveals a degree of distortion in renal structure when compared with the control group.

Table 3. Effect of Administration of fraction 3 of Ethanol leaf extract of *Ziziphus muritiana* on haematological parameters of wistar rats.

Parameters	*Ziziphus muritiana Ethanol* leaf extract fraction 3 (mg/kg body weight)		
	Control	**200**	**500**
Haemoglobin (g/L)	14.92 ± 0.066[a]	14.04 ± 0.652 [a]	10.62 ± 1.061 [b]
Red blood cell ($\times10^{12}$/L)	7.96 ± 0.086 [a]	7.59 ± 0.295 [a]	7.44 ± 0.126 [a]
Packed cell volume (%)	53.37 ± 0.220 [a]	51.09 ± 3.190 [a]	42.60 ± 1.973 [b]
Mean Corpuscular Haemoglobin (pg)	19.00 ± 0.251 [a]	18.91 ± 0.436 [a]	17.24 ± 1.591 [a]
Mean Corpuscular Haemoglobin Concentration (%)	28.21 ± 0.064 [a]	27.60 ± 0.422 [a]	26.50 ± 1.642 [a]
Mean Corpuscular Volume(fl)	66.69 ± 0.554 [a]	68.71 ± 1.867 [a]	61.15 ± 2.203 [b]
White Blood Cell ($\times10^9$ /L)	14.38 ± 0.829 [a]	7.64 ± 0.248 [b]	6.25 ± 0.942 [b]
Lymphocytes ($\times10^9$/L)	84.55 ± 1.554 [a]	38.77 ± 2.237 [b]	51.79 ± 9.595 [b]
Neutrophils ($\times10^9$/L)	32.0± 1.82[a]	32.0± 1.82[a]	32.0± 1.82[a]
MID (%)	8.00 ± 0.55 [a]	8.40 ± 0.25 [a]	8.20 ± 0.49 [a]
Platelets ($\times10^9$/L)	7.91 ± 0.370 [a]	5.21 ± 0.484 [b]	5.24 ± 0.068 [b]

Note: MID is the average of monocytes, eosinophils, basophils. N = 5, X ± SEM.

[a-c] test values carrying superscripts different from the control across each parameter are significantly different at $P > 0.05$.

Table 4. Effect of administration of Ethanol leaf extract fraction 3 of *Ziziphus muritiana* on some Kidney Function Indices in wistar rats.

Parameters	*Ziziphus muritiana* ethanol leaf extract (mg/kg body weight)		
	Control	**200**	**500**
Creatinine (umol/L)	0.047 ± 0.002[a]	0.043 ± 0.006[a]	0.098 ± 0.124[a]
Urea (mmol/L)	339.370 ± 14.034[a]	353.890 ± 60.211[a]	462.890 ± 13.002[a]
Sodium (mEq/L)	136.50±1.63 [a]	138.8± 1.59[a]	140.83± 1.534 [a]
Calcium (mg/dL)	6.61 ± 0.12 [a]	6.40 ± 0.56 [a]	6.94 ± 0.24 [a]
Potassium(mEq/L)	5.19 ± 0.25 [a]	6.39 ± 0.50 [a]	9.57 ± 1.37 [b]
Chloride (mEq/L)	96.00 ± 0.35 [a]	88.27 ± 1.89 [b]	92.88 ± 0.49 [b]

N = 5, X ± SEM.[a-c] test values carrying superscripts different from the control across each parameter are significantly different at P > 0.05

Brine Shrimp Lethality Assay of subfractions of fraction 3 of ethanol leaf extract of *Z. mauritiana*

The preparative thin layer chromatography of column pooled fraction 3 which resulted in three (3) sub-fractions was evaluated by brine shrimp lethality assay. The sub-fractions of column pooled fraction II are shown in Table 5. The result revealed that sub-fraction two (2) gave the lowest lethal concentration of 446.68µg/ml (0.45 mg/ml) followed by fraction three (3) and fraction one (1) with a lethal concentration of 793.78µg/ml (0.79 mg/ml) and 2390.01 µg/ml (2.39 mg/ml) respectively.

Table 5. Brine Shrimp Toxicity of sub fractions of ethanol leaf extracts fraction III of *Z. mauritiana*.

Extract	Dose (µg/ml)	Percentage Mortality	LC_{50} (µg/ml)	Toxicity Classification
Fraction 3:1	0	0	2390.01	Not Toxic
	10	16.67		
	100	30.00		
	1000	93.33		
Fraction 3:2	0	0	446.68	Toxic / Moderately toxic
	10	13.00		
	100	32.00		
	1000	52.45		
Fraction 3:3	0	0	793.78	Toxic /Slightly toxic
	10	33.00		
	100	63.00		
	1000	96.67		

Liquid Chromatography –Mass Spectrophotometry of sub-fraction II of column pooled Fraction III (3) of Ethanol leaf extract of *Zi*ziphus *mauritiana.*

The sub-fraction II of column pooled fraction 3 obtained by preparative thin layer chromatography (TLC) was analyzed for its bioactive composition using Liquid Chromatography –Mass Spectrophotometery (LC-MS) and the constituents were presented in Table 6. The analysis revealed the presence of a range of phytoconstiruents such as alkaloids, glycosides, triterpenoids, steroids, and flavonoid derivatives. The most abundant bioactive molecules are glycoside derivatives with Centellasaponin C as the most abundant.

Table 6. Chemical constituents of Sub-fraction 2 of column pooled fraction 3 of ethanol extract of *Ziziphus mauritiana* leaf.

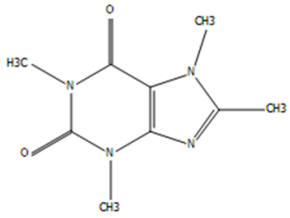
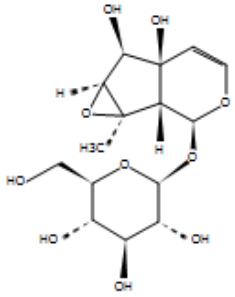
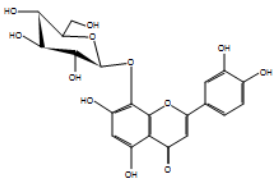
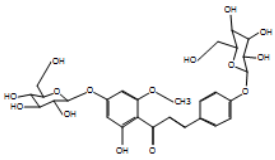
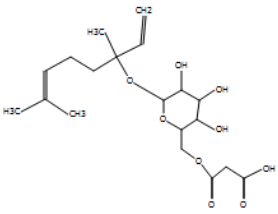
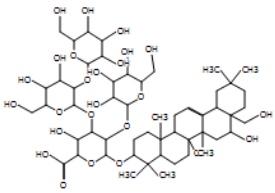
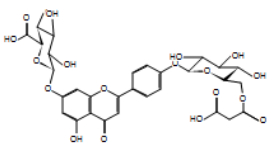
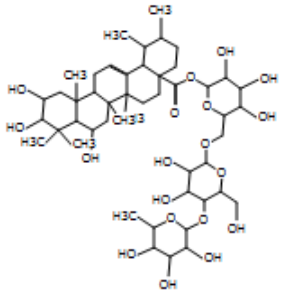
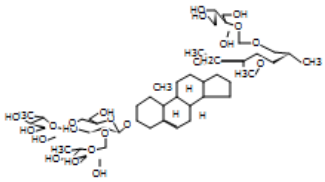
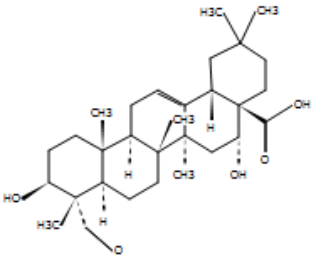
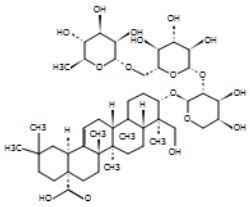
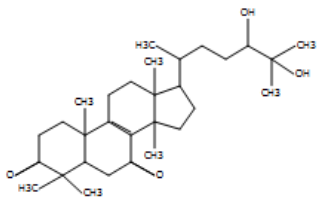
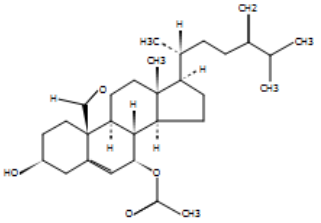
S/N	Class of Phytochemicals	Compound Name	Structure	Activity	References
1	Alkaloids	8- Methylcaffeine		Metabolite of caffeine, Cause abbereration in allium ceppa, cell culture Aberration occurred in late prophase and Late G2 phase	Kihlam *et al.*, 1972 Jiang *et al.*, 2000
2	Glycoside	Antirrhinoside		An irridiod glycoside Haemolytic activity Cytotoxic activity	Drohse and Molgaard, 2001 Tholl *et al.*,2004 Riaz *et al.*, 2013
3		8-hydroxyluteolin 8-glucoside		Flavone glycoside Induce DNA Damage	Cantero *et al.*, 2006, Snyder and Gillies, 2002
4	Glycoside	4,2',4'-Trihydroxy-6'-methoxychalcone 4,4'-di-beta-glucoside		Flavon-glycoside, induce DNA damage	Lopez-lazaro, 2009, Kawanishi, 2000. Snyder and Gillies, 2002
5		D-Linalool 3-(6"-malonylglucoside)		Glycoside, Derivative of D-linalool which is reported for genotoxic activity and dermatological allergy	Api *et al.*, 2015, Nakamura *et al.*, 2009
6		Camellioside D		Triterpene oligoglycoside Antiproliferative activity	Guo *et al*, 2018, Nakamura *et al.*, 2012
7		Apigenin 7-glucuronide-4'-(6"-malonylglucoside)		A glycoside, derivative of Apigenin,cytotoxic activity by DNA damage	Budhraja et al., 2012 McGaw *et al.*, 2013

Table 6. *Cont.*

S/N	Class of Phytochemicals	Compound Name	Structure	Activity	References
8	Saponin	Centellasaponin C		Triterpene Saponin Hepatotoxic	Jorge and Jorge, 2005
9		Dioscoreside C		Steroidal Saponin Cytotoxic activity	Dong *et al.*, 2001 Hansakul *et al.*, 2008
10		Quillaic acid		Aglycone Saponin, cytotoxic activity, adjuvant effect	Rajput *et al.*, 2007, Gevrenova *et al.*, 2014
11	Triterpenoid	Akeboside stf		Triterpenes Cytotoxic Activities	Chudzik *et al.*, 2015 Kolesnikova *et al.*, 2013
12	Steroids	Lucidumol A		Triterpenoid steroids Cytotoxic activity	Xia *et al.*, 2014 Amen *et* al., 2016
13		Nebrosteroid L		Steroids *cytotoxic activities*	Amir *et al.*, 2012

DISCUSSION

Effect of Different column Pooled Fractions of Ethanol extract of *Z. mauritiana* leaves

The column chromatography separation of crude ethanol extract of *Z. mauritiana* leaves yielded 7 (seven) distinct column pooled fractions with Fraction 1 as the highest yield (Table 17), this may be due to the degree of solubility of phytochemicals present in fraction I. Different phytochemicals exhibit different degrees of solubility in solvent systems (Wakeel et al., 2019) Fraction 3 was observed to be the most bioactive component of the extract fractions using brine shrimp

lethality assay (BSLA) techniques (Table 18) (Ode *et al.*, 2012). This effect may be due to the presence of some bioactive principles that have been reported to be toxic (Benie, *et al.,* 1990).

Effect of column Pooled Fraction Three (3) of Ethanol extract of *Z. mauritiana* leaves

Fraction three was picked to be the most toxic fraction based on the Brine Shrimp lethality Assay (BSLA) carried out to determine the effect of the bioactive component of the sub-fraction on the experimental model (*artemia Salina* Larve). Subchronic toxicity evaluation of plant extracts is used to determine its effect on blood and biochemical parameters. Also, morphological investigation on specific tissues may help to explain the mechanisms of toxicity of an extract and its possible therapeutic effect (Yamthe *et al.,* 2012, Asaduzzaman et al., 2015).

The main objective of determining hemoglobin concentration and its indicative parameters (MCH and MCHC) is to analyze the amount of intracellular iron and an index for folic acid and vitamin B12 need, also oxygen carrying capacity of the blood while the primary reasons for assessing the RBC, PCV, and MCH is to check anemia, evaluate normal erythropoiesis and degree of anemia or polycythaemia (Ganong, 2001). The reduction observed may be an indication of the inability of the blood to perform the function of oxygen-carrying capacity which leads to a decrease in energy production within the cells (Asanga *et al.,* 2013), and may be responsible for the weakness/slow activity observed during extract administration to the Wistar rats. Increased total WBC count upon administration of xenobiotics from either plant extract or synthetic compound may indicate leucocytosis in the treated animals (Ajeigbe *et al.,* 2013), and evaluating differential WBC count provides information on the proportion of the different white cells present in circulating blood (Cheesbrough, 2000; Enitan *et al.,* 2012). The observed significant increase in counts of lymphocytes, neutrophils, monocytes, and eosinophils in the test groups when compared to the control is an indication that the extract must have induced lymphocytosis, neutrophilia, monocytosis, and eosinophilia respectively (Ajeigbe *et al.,* 2013) but he reduction in these parameters may indicate immunosuppression of the defense mechanism of the animal and toxicity of the extract may be evident. The observed reduction in the concentration of WBC and Lymphocytes in the blood sample of all the treated groups with fraction 3 when compared with the control group suggests the immunosuppressive ability of the extract fraction when ingested. Also, reduced platelet concentration has been reported to increase blood viscosity and lead to increased blood pressure (Adedapo *et al.,* 2008; Adeniyi *et al.,* 2010). The reduction observed in platelet count may be an indication of an increase in the blood pressure of the animal therefore certain phytoconstituents of administered extract fraction may induce high blood pressure.

Biochemical evaluation using enzymatic and non-enzymatic indices/parameters has been used to evaluate the functionality and integrity of cellular barriers of vital tissues such as the liver and kidney (Sunmonu *et al.,* 2014). These parameters provide information on the effect and nature of pathological damage that may have taken place in these tissues (Yang *et al.,* 2014).

Elevated serum ALT, AST, and ALP concentrations in the serum have been reported in hepatic injury and diseases (Andrade *et al.,* 2006, Stirnimann *et al.,* 2010; Owolarafe *et al.,* 2020), increased concentrations of ALT, AST, and ALP in the serum upon administration of fraction 3 of ethanol extract of *Z.mauritiana* when compared the control group ($P < 0.05$) suggested severe mixed hepatic injury such cholestatic injury, bile duct injury and overall distortion of metabolic homeostasis of the body (Kaplowitz, 2005; Stirnimann *et al.,* 2010, Yang *et al.*,2014). This is corroborated by increased MDA concentration observed in treated groups which is an indication of membrane lipid peroxidation and a compromise of hepatic cell membrane integrity.

Nephrotoxicity is the adverse effect observed on the function of the renal tissue that occurs due to the intake of xenobiotics resulting in direct toxicity or compromised renal perfusion, these renal dysfunctions may include acute tubular necrosis, glomerular and tubulointerstitial injury, haemodynamically mediated damage, and obstructive nephropathy (Finlay *et al.,* 2013; Taber and Mueller, 2006). No significant increase or reduction in serum creatinine, urea, and electrolytes concentrations in all treated groups when compared with the control ($P < 0.05$) may indicate the non-nephrotoxic effect of fraction 3 of ethanol extract of *Z.mauritiana leaves* in rats.

Amresh *et al.,* (2008) suggested that Organ to body weight ratio may indicate organ swelling, atrophy, or hypertrophy and The histopathological analysis of tissue biopsies is the most definitive way to diagnose and confirm various types of impairment in tissues (Yang *et al.,* 2014); The increase in liver, kidney, and lung to body weight ratio following the administration of fraction 3 of ethanol extract of *Z.mauritiana leaves* in rats may be a result of inflammation. This submission is in agreement with earlier reports by Ashafa *et al*. 2011 and Adebayo *et al.,* 2003. Histopathological examination also reveals the presence of inflammation and distortion of the cellular architecture of the liver and Lung tissue and a mild atrophy of the kidney.

CONCLUSIONS

Base on the findings in this research work the extract exhibited mild toxicity and these maybe due to identified phytochemicals and their antagonistic and synergistic activities with regards to the identified bioactive compounds

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Authors' Contributions: Tajudeen A. Owolarafe: Is the lead researcher and participate in all phase of the research from conceptualization to writing of the manuscript. Salawu Kailani: He is involved in the fractionation of crude extracts and writing of the manuscript

Competing Interests:

Ethics approval and consent to participate: Ethical approval was obtained from the research ethical committee of Bayero University Kano with approval number BUK/CHS/REC/68.

Consent for publication: The authors declare that there are no competing interests.

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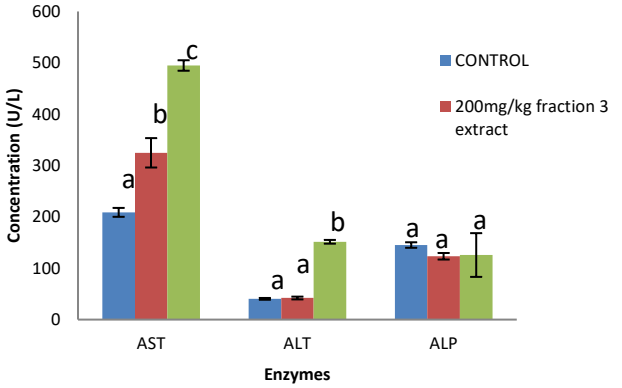

Figure S1. Effect of Administration of fraction three of ethanol extract of *Zizphus mauritiana* leaves on Liver Function enzymes Activities among experimental animals.

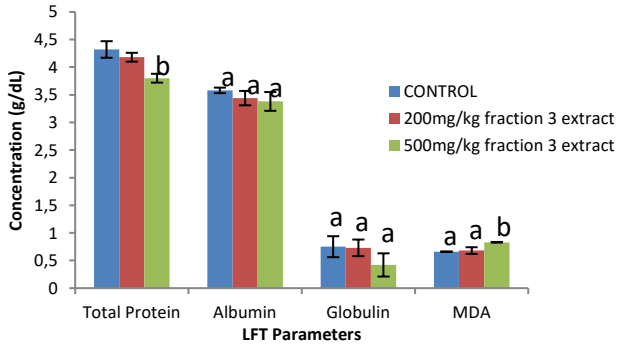

Figure S2. Effect of Administration of fraction three of ethanol extract of *Zizphus mauritiana* on Liver function biochemical paramters among experimental animals.

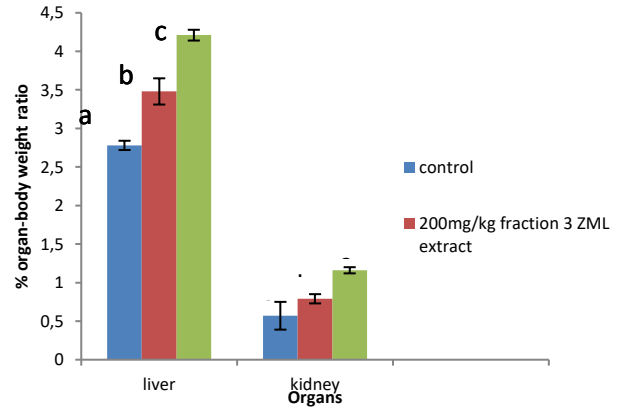

Figure S3. Effect of oral administration of fraction III of ethanol extract of *Z. mauritiana* leaf on organ/body weight ratio.

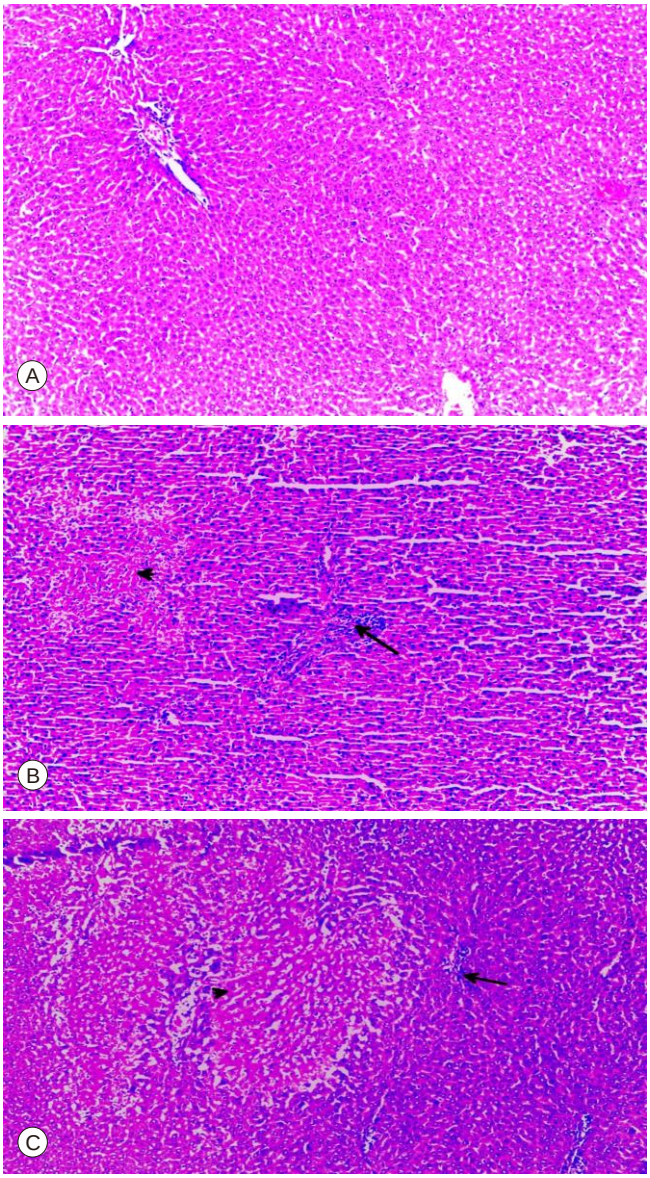

Figure S4. Photomicrographs cross section of Liver from wistar rats administered with distilled water (A), 200mg/kg fraction 3(B) and 500mg/kg fraction 3 (C) of Ethanol leaf extract of Z. orally for 14 days (Group 1) (X 100) haematoxylin and eosin.

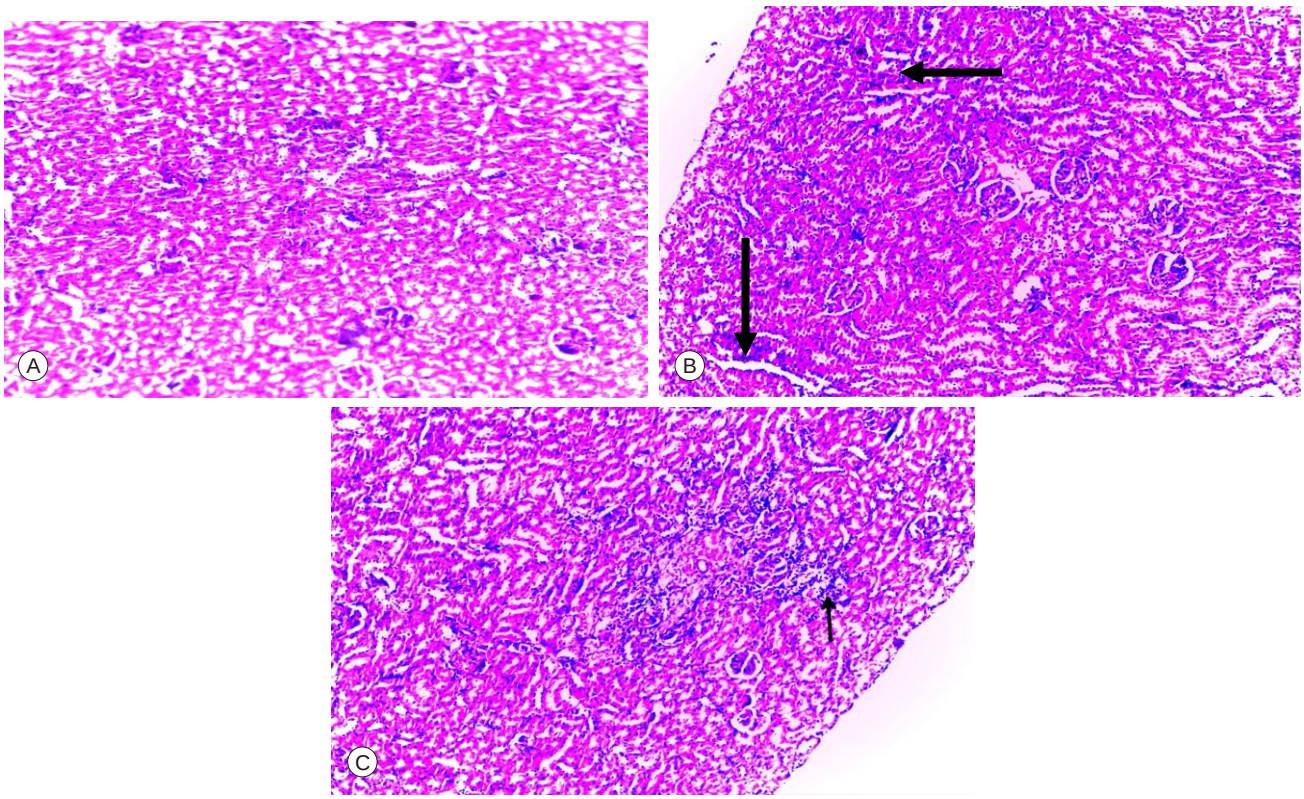

Figure S5. Photomicrographs cross section of Kidney from wistar rats administered with distilled water (A), 200mg/kg fraction 3(B) and 500mg/kg fraction 3 (C) of Ethanol leaf extract of Z. orally for 14 days (Group 1) (X 100) haematoxylin and eosin.