Niger. J. Physiol. Sci. 37 (December 2022): 235 – 246
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full length Research Article

Haematoprotective and Hepatoprotective Effects of Methanolic Leaf Extract of *Parquetina nigrescens* on Arsenic trioxide-induced Toxicity in Male Wistar Rats

Daramola O.O.[1], Oyeyemi W.A.[2], Akinola A.O.[3], Raji Y.[4].

[1]*Department of Physiology, Redeemer's University, Ede, Osun State, Nigeria.*
[2]*Department of Physiology, College of Health Sciences, Osun State University, Osogbo, Nigeria.*
[3]*Department of Physiology, Ondo University of Medical Sciences, Ondo City, Ondo State, Nigeria.*
[4]*Laboratory for Reproduction and Developmental Programming, Department of Physiology University of Ibadan, Ibadan, Nigeria.*

Summary: This study was designed to investigate the haematoprotective and hepatoprotective effects of methanol extract of *Parquetina nigrescens* leaf (MEPL) on arsenic trioxide-induced toxicity in male Wistar rats. Phytochemical screening, in vitro antioxidant assay and gas chromatography-mass spectrometry (GC-MS) were determined on the MEPL. Six female Wistar rats were used for LD50 study. Forty male Wistar rats were grouped into eight and orally treated for 54 days as follows: Group 1 (10% $_{tween}$ 80), Group 2 (3 mg/kg As_2O_3) Groups 3, 4 and 5 (250, 500 and 1000 mg/kg MEPL) and groups 6, 7 and 8, (250 mg/kg+ As_2O_3, 500 mg/kg+As_2O_3 and 1000 mg/kg+As2O3). The animals were sacrificed on day 55 under anaesthesia. Blood was collected by cardiac puncture for heamatological studies and liver tissue was harvested for spectrophotometric determination of malondialdehyde (MDA) concentration, superoxide dismutase (SOD), aspartate aminotransferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) activities. Liver histology was also assessed. Flavonoids, tannin, alkaloids, saponin, and anthraquinone were present in MEPL, also, MEPL scavenged 2,2 diphenyl-1-picrylhydrazyl hydrate (DPPH) and Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid radical (ABTS+). The IC50 of MEPL required to chelate metal was also low. The GC-MS revealed the presence of 24 essential oil. The LD50 was greater than 5000 mg/kg. White blood cell count and SOD activity reduced ($p<0.05$) in 3 mg/kg As_2O_3 group when compared with control but significantly increased in groups co-treated with AS_2O_3 and 250 mg/kg, 500 mg/kg or 1000 mg/kg MEPL. Packed cell volume and red blood cell count were significantly reduced in 1000 mg/kg MEPL group when compared with the control group. MDA concentration, AST, ALT and ALP activities increased significantly in As_2O_3 only group but decreased ($p<0.05$) in the groups treated with 250 mg/kg+As2O3, 500 mg/kg+As2O3 and 1000 mg/kg As_2O_3 when compared with the control group.

Keywords*: Parquetina nigrescens leaf, arsenic trioxide, redox status, antioxidant, liver, blood*

**Authors for correspondence: daramolaoore@run.edu.ng; Tel: +2348038169338*

Manuscript received- August 2022; Accepted- October 2022

DOI: https://doi.org/10.54548/njps.v37i2.11

INTRODUCTION

Human activities ranging from agriculture to mining and smelting of non-ferrous metals have uncovered many elements such as cadmium, lead and arsenic among others which are abundantly present in the earth crust (Florea *et al*., 2004). These elements have been found useful in preservation of agricultural products, production of ammunitions, car batteries, production of colourless glass and in fact, drugs (NRC, 1999; Scheindlin, 2005). Despite their usefulness, arsenic and other heavy metals have been associated with hazardous effects on man (Bradl, 2002). Arsenic is present in surface water and underground water in some geographical areas such as Ugbe and Akoko area of Ondo state and in Ibadan, Oyo state in Nigeria (Afolabi *et al*., 2011; Egbinola and Amanambu, 2014) as well as in some countries in Asia such as Bangladesh, Taiwan and China and therefore ingested through drinking water (Ahoule *et al*., 2015). It is also present in soil and absorbed by food crops like rice (Nriagu and Azcue, 1990). More importantly, people who live in these areas are exposed to arsenic either through inhalation, skin contact or ingestions in their various places of work where arsenic is used (ASTDR, 2007).

Arsenic is of two types; the trivalent arsenic which is very toxic because of its ability to cross the cell membrane and get accumulated in the cells when cellular antioxidant system is depleted (Bergquist *et al*., 2009) and the pentavalent arsenic which is less toxic because it can hardly penetrate cell membrane (Cullen and Reimer, 1989). Arsenic trioxide (AS2O3) belongs to the class of trivalent arsenic. Chronic exposure to As_2O_3 has detrimental effects on health as it causes reduced white blood cell (WBC) count (Kyle and Pearse, 1965; Kannan *et al*., 2001), depletes antioxidant enzymes and causes hepatotoxicity (Cohen *et al*., 2006; Yasmin *et al*., 2011). Adverse effects of As_2O_3 are

usually treated with chelators such as 2,3-dimercapapto-1-propanesulfonate (DMPS) (Flora *et al*., 2007). Chelators also bring about the excretion of other metals such as sodium, calcium, zinc, colbat, magnesium, and molybdenum that are of significant importance to normal body functions. Sodium ion is needed for excitability of cell membrane as well as for its participation in facilitated transport of nutrients and other ions (Constantin *et al*., 2011). Calcium ion is required for neurotransmitters to be released at nerve junctions, maintainance of bone and teeth integrity and for depolarization of cardiomyocytes (Pravina *et al*., 2013). Zinc ion is necessary for many enzymatic/catalytic functions and also acting as gene transcription factor (Bhowmik, *et al*, 2010). Hence the need to search for other methods of protecting the body from toxic effects of arsenic.

In African tradition, there is so much reliance on common herbs for food, healing and prevention of diseases owing to their rich phytochemical constituents and chemical compounds. One of such plants is *Parquetina nigrescens*. *Parquetina nigrescens* is a plant that grows well in East and West Africa (Iwu, 1993). It is a twigging and herbaceous plant; its leaf is usually 6-8 cm wide and 10-15 cm long. It is used in traditional medicine to boost blood production, combat fatigue, cure headache, stomach ulcer and insanity (Iwu, 1993; Datte *et al*., 1996; Erah *et al*., 2003; Alvarez, 2012). Folkloric use of its leaf in the treatment of anaemia (Omoboyowa *et al*., 2016) as well as its antioxidative property (Ayoola *et al*., 2011) have been validated. The potency of *Parquetina nigrescens* leaf is dependent upon its phytochemical composition. It was therefore pertinent to study its phytochemical composition. Furthermore, in the event of hepatotoxicity caused by exposure to arsenic, the possible outcome with the intervention of *Parquetina nigrescens* leaf extract is yet unknown. Therefore, this work was designed to study the phytochemical composition of methanol extract of *Parquetina nigrescens* leaf and its effects on arsenic trioxide-induced hepato-toxicity in male Wistar rats.

MATERIALS AND METHODS

Plant material and extract preparation: Fresh leaves of *Parquetina nigrescens* were hand-picked within the premises of University of Ibadan, Ibadan. The whole plant of *Parquetina nigrescens* was authenticated at the Forestry Research Institute of Nigeria (FRIN) and given FHI number - 109785. The harvested leaves were left to dry at room temperature for about six weeks and blended to powdery form with Daewoo electrical blender (DBL-819, Posco Daewoo Corporation, Korea). The weight of the *Parquetina nigrescens* powder was determined (about 1100 g). Using absolute methanol, Soxhlet extraction method was employed for the extraction process (Aderibigbe *et al*., 2011). The methanol extract of *Parquetina nigrescens* leaf (MEPL) was concentrated using rotary evaporator at 40°C and thereafter, freeze dried to obtain a powdery form. The methanol extract of *Parquetina nigrescens* leaf weighing 104 g was obtained and stored at room temperature throughout the experiment.

Phytochemical study of methanol extract of *Parquetina nigrescens* leaf

Test for alkaloids: Methanol extract of *Parquetina nigrescens* leaf (500 mg) was dissolved in 5 mL of methanol and mixed with 5 mL of 1% aqueous hydrochloric acid over water bath, then 1 mL of the solution was added to few drops of Mayer's reagents. Fluorescent brownish precipitate indicates the presence of alkaloids (Edeoga *et al.,* 2005).

Test for saponin: Methanol extract of *Parquetina nigrescens* leaf (500 mg) was mixed with distilled water and persistent frothing when warmed showed that saponin was present. Further, the supernatant boiled with 50 mL phosphate buffer and was passed through an asbestos disc already soaked with cholesterol in ether. The disc was washed, dried, boiled in 20 mL of oxylol and then washed in ether and placed on a 7 % blood nutrient agar. Complete haemolysis of red blood cells around the disc after 6 hours confirms the presence of saponin (Edeoga *et al.,* 2005).

Test for tannin: The presence or absence of tannin was established in line with Trease and Evans (1989). Methanol extract of *Parquetina nigrescens* leaf (5 g) was mixed with 10 mL of distilled water and filtered. Ferric chloride reagent was added to the filtrate. A blue-black, green or blue-green precipitate indicates the presence of tannins.

Test for anthraquinone: The MEPL (5 g) was mixed with 10 mL of benzene and filtered. To the filtrate, 10% ammonia solution was mixed, and development of pink colour confirms the presence of anthraquinones (Edeoga *et al*., 2005).

Test for flavonoids: To 3 mL of MEPL, magnesium ribbon and 1 mL of concentrated HCl was added. Appearance of red colour verify presence of flavonoid (Kumar *et al*., 2007).

Test for cardiac glycosides: Test for cardiac glycosides is also known as Kellar–Kiliani test and it was carried out according to the procedure of Parekh and Chanda (2007). To 2 mL of MEPL filtrate, 1 mL each of glacial acetic acid, ferric chloride and concentrated sulphuric acid were added. Change in the mixture colour to green-blue indicates the presence of cardiac glycosides.

***In vitro* antioxidant study**

Metal chelating ability assay: The ferrous ion-chelating (FIC) assay was done according to the method of Singh and Rajini (2004). Solution of 2 mM $FeCl_2 \cdot 4H_2O$ and 5 mM ferrozine were diluted 20 times. An aliquot (1 ml) of different concentrations of MEPL was mixed with 1 mL $FeCl_2 \cdot 4H_2O$ and incubated for 5 minutes. To the mixture, 1 mL of ferrozine was added, and mixed vigorously, and incubated for 10 minutes. The absorbance was measured at 562 nm. Ethylenediaminetetraacetic acid (EDTA) was used as the standard drug.

2,2-diphenyl-1-picrylhydrazyl hydrate assay: 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH), a deep violet coloured substance that reduce on reaction with an antioxidant compound that can give off hydrogen and have

its colour changed to light yellow (Blois, 1958), this test was done as described by Brand-Williams *et al.* (1995). The DPPH dissolved in methanol (0.3 mM) was added to different concentrations of MEPL and the standard which is vitamin C and incubated for 30 minutes. The absorbance was taken at 517 nm against DPPH control and the percentage of inhibition was calculated.

Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid radical scavenging activity: The free radical scavenging activity of MEPL was determined by the ABTS radical cation decolourisation assay (Re *et al*., 1999). ABTS working solution (2 mL) was mixed with 50 µL of different concentrations of MEPL or trolox which served as the standard and the absorbance was measured after 20 minutes at 734 nm.

Determination of total flavonoid content: Standard quercetin with varying concentration was used as standard in comparison to MEPL. This was carried out based on the aluminium chloride colorimetric assay as described by Zhilen *et al.* (1999) and Miliauskas *et al.* (2004). Distilled water, Aluminium chloride and sodium hydroxyide were added to MEPL and. The absorbance was measured against blank at 519 nm. The total flavonoid content of the plant was expressed as mg quercetin equivalents per gram.

Determination of total phenol content: Total phenol content was determined according to the procedure of Slinkard and Singleton (1977). A mixture of standard (gallic acid) or MEPL (0.1 mL), water (5 mL) and 0.5 mL of 0.2 N Folin-Ciocalteu's phenol reagent was vortexed After 3 minutes, 1.5 mL of 7% (w/w) Na_2CO_3 solution was added, vortexed and incubated for 2 hours at room temperature. Its absorbance was then measured against blank at 750 nm. 0.1 mg/mL of Gallic acid was used as standard.

The principle of determining total antioxidant capacity (TAC) is centred on the reduction of molybdenum (VI) to molybdenum (V) by the sample and the subsequent formation of a green phosphate/molybdenum (V) complex at an acidic pH (Prieto *et al.,* 1999). A mixture of MEPL or standard solutions of ascorbic acid and the reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was incubated in a water bath at 95^{o}C for 90 minutes, cooled to room temperature, and its absorbance was measured at 695 nm. The total antioxidant activity was expressed as an ascorbic acid equivalent (AAE).

Gas chromatography-mass spectrometry: The GC-MS analysis of MEPL was performed as described by Karasek and Clement (1988) using a Perkin Elmer GC–MS equipped with a VF-5 MS fused silica capillary column (30 m × 0.25 i.d., film thickness 0.25 µm) (Model Perkin Elmer Clarus 500, USA) at the central laboratory, University of Lagos. An electron ionization system with ionization energy of 70 eV was used for the detection of the chemical compounds. Helium gas with purity 99.999% was used as a carrier gas at constant flow rate of ±1 mL/min. Mass transfer line and injector temperature were set at 220 and 290 °C, respectively. The temperature program was set for oven from 50 to 150 °C at 3 °C/min, then held isothermal for 10 minutes and finally raised to 250 °C at 10 °C/min. The extract was diluted with methanol solvent into 100 folds and 1 µl of the diluted sample was injected in the split mode with split ration 120:1. The delay time was 2 minutes, and the total running time was 75 minutes (the retention time was between 9 and 29 minutes). The relative percentage of the chemical compounds in MEPL was expressed as area percentage. The chromatogram was interpreted using National Institute of Standards and Technology (NIST) library consisting two hundred thousand.

Experimental animals: Forty-six Wistar rats; 6 females and 40 males (150 – 180 g) were used. They were obtained from the Central Animal House, College of Medicine, University of Ibadan, Ibadan. They had free access to rat feed and drinking water *ad libitum*. All animals were distributed into groups and acclimatized for two weeks. All experiments on animals were carried out with the approval of University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC) and the number - UI-ACUREC/18/0067 was assigned.

Experimental design

LD_{50} test: The LD_{50} was done using limit test as described by Organization for Economic Co−operation and Development (OECD) guideline 423 (2001). Six healthy and non-pregnant adult female rats were used for acute toxicity test. They were grouped into two, each group consisting of three animals. The first group was administered 2,000 mg/kg bw of MEPL and observed for fourteen days. The second group was also administered the same dosage of MEPL and observed for 14 days.

Experimental groups: The animals used for this experiment were randomly distributed into 8 groups of five animals each. The animals were treated with MEPL and/or arsenic trioxide orally for 54 days as follows: group 1 (control) was treated with 10% Tween 80, group 2 was treated with 3 mg/kg AS_2O_3, groups 3, 4 and 5 were treated with 250 mg/kg MEPL, 500 mg/kg MEPL and 1000 mg/kg MEPL respectively, groups 6, 7 and 8 were treated with 250 mg/kg MEPL + As_2O_3, 500 mg/kg MEPL + As_2O_3 and 1000 mg/kg MEPL + As_2O_3 respectively. The dosage of Arsenic trioxide used was adopted from Alli *et al*. (2013). Tween 80 (10%) was used to dissolve MEPL.

Sacrifice and collection of organs: After 54 days of oral treatment with MEPL and arsenic trioxide, all animals were anaesthetized using 40 mg/kg of intraperitoneal injection of sodium thiopental (Shittu *et al*., 2018) and blood was collected by cardiac puncture into EDTA bottles. Liver was harvested, weighed, and used for further laboratory studies.

Blood indices

Determination of packed cell volume and haemoglobin concentration: Packed cell volume (PCV) was determined by filling heparinized capillary tube with blood up to 75% of its total volume, sealed with plasticine and centrifuged at 12,000 rpm. It was then read off with haematocrit reader and expressed in percentage.

The haemoglobin concentration in grams per deciliter (g/dL) of blood was determined by multiplying PCV with 0.34.

Determination of Red blood cell count: Red blood cell (RBC) count was determined by filling a diluting pipette for RBC with blood up to 0.5 mark and with blood diluting fluid up to 101 mark and mixed. The mixture was allowed to fall to the 100 mark and a Neubauer's counting chamber was charged with 2-3 drops after putting the cover slide in place. The RBC in 5 selected squares were counted under the microscope by applying Thoma rule. The number of RBC counted was multiplied by 10,000 and expressed as $10^6/\mu L$.

White blood cell count and platelet count: White blood cell (WBC) count and platelet count were determined by filling a pipette with blood up to 0.5 mark and with Turks solution up to the 11 mark and allowed to mix. A Neubauer's counting chamber was charged with 2-3 drops after putting the cover slide in place The WBC were counted in the four large squares and the total number was multiplied by 200 while platelets were counted in the depressed small squares on the same chamber.

Biochemical assay: Liver (1 g) was weighed and rinsed with 1.15% of KCl and blotted with filter paper. Each one was then homogenized with 5 ml of phosphate buffer saline (pH 7.4) using Teflon homogenizer and centrifuged at 10,000 rpm for 10 minutes. The obtained supernatant was used for the estimation of malondialdehyde (MDA) concentration superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), alkaline phosphatase (ALP), alanine aminotransferase (ALT) as well as aspartate aminotransferase (AST) activities.

Malondialdehyde: The reaction between thiobarbituric acid and MDA in a sample produces red colour that can be measured spectophotometrically. The procedure designed by Rice-Evans *et al*. (1986) was adopted. The liver supernatant (1 mL) was mixed with 2 mL of trichloroacetic acid-thiobarbituric acid-hydrochloric acid and heated in water bath for 15 minutes. It was cooled, centrifuged and the absorbance of the supernatant was measured at 535 nm. The result was expressed as nM/mg tissue.

Superoxide dismutase: The presence of SOD in a sample prevents the rapid auto-oxidation of adrenaline to adrenochrome. The concentration of adrenochrome can be measured at 420 nm. The method of Misra and Fridovich (1972) was used for the estimation of SOD. The liver supernatant (0.2 mL) was mixed with 2.5 mL of carbonate buffer and equilibrated at room temperature. Thereafter, 0.3 mL of adrenaline solution was added to it and the absorbance was obtained.

Catalase: The presence of catalase in a sample causes peroxidative action which leads to giving off oxygen that is observed in the form of bubbles. The activity of catalase was determined by the procedure of Sinha (1972). The liver supernatant (0.5 mL) was reacted with 5.0 mL of hydrogen peroxide, 1.0 mL of tetraoxosulphate (VI) and 7.0 mL potassium permanganate. Absorbance was read at 480 nm against distilled water after 30 second and after 60 seconds.

Aspartate amino transferase: Aspartate amino transferase (AST) catalyzes the formation of oxaloacetate hydrozone that can be monitored at 340 nm when L-aspartate, L-oxoglutarate and 2,4-dinitrophenylhydrazine are reacted with a sample (0.1 mL of liver supernatant). The modified method of Reitman and Frankel (1957) was adopted.

Alanine amino transferase: The presence of alanine amino transferase (ALT) in a sample (liver supernatant) when reacted with L-alanine, α-oxoglutarate and 2,4-dinitrophenylhydrazine catalyzes the production of pyruvate hydrozone and the rate of change in absorbance at 340 nm can be observed over a fixed time interval. The study was done according to the modified procedure of Reitman and Frankel (1957).

Alkaline phosphatase: Alkaline phosphatase acts upon the 2-Amino-2-Methyl-1-propanol-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and at the same time, brings about the development of blue chromogen, which is measured photometrically at 590 nm.

Histology of the tissues: The liver tissue was fixed in 10% formalin for minimum of five hours before the commencement of tissue processing which involved the processes of dehydration with different percentages of alcohol; clearing using xylene; embedding in paraffin wax; trimming; nicking and sectioning with a microtome and lastly, staining of the tissues. Thereafter, pictures of the processed tissue slide were taken for proper observation of morphological changes.

Statistical analysis

Data from each group were expressed as mean ± standard error of mean (mean ±SEM). With the aid of Statistical Package for Social Sciences (IBM SPSS Statistics 25), data collected were analysed using one-way analysis of variance followed by Waller-Duncan's post-hoc test. The p-value was set at less than 0.05 to determine the level of significance.

RESULTS

Phytochemical constituents of methanol extract of *Parquetina nigrescens* leaf: The following plant phytochemicals: alkaloids, tannins, saponins, flavonoids and anthraquinones were present in methanol extract of *Parquetina nigrescens* leaf (Table 1).

Table 1.
Phytochemical constituents of methanol extract of *Parquetina nigrescens* leaf

Phytochemical Constituent	Present or Absent
Flavonoids	++
Tannins	++
Saponins	++
Alkaloids	++
Anthraquinones	+
Phytostetrol	-
Glycosides	-
Cardiac glycosides	-

Key:
++ Presence of detectable quantity
+ Suspected presence
- Absent

Table 2:
Comparison of *in vitro* antioxidant capacity between standard drugs and MEPL

	IC_{50} of Metal chelating ability (µg/mL)	**IC_{50} of 2,2-diphenyl-1-picrylhydrazyl scavenging activity (mg/mL)**	**IC_{50} of Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid radical scavenging activity (mg AAE/g)**	**Flavonoid content (mg QE/g)**	**Phenol Content (mg/GAE.g)**
Standard	0.154±0.02	0.05 ± 0.002	5.02 ± 0.1	5.02 ± 0.1	4.38 ± 0.1
MEPL	1.42 ± 0.01	1.54 ± 0.06	10.34 ± 0.24	7.32 ± 0.19	2.71 ± 0.09

Table 3:
Chemical formula and name of compounds detected in MEPL

Pk#	RT	Area%	Chemical Formula	Library/ID
1	9.024	3.09	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol
2	10.185	1.14	$C_7H_{14}O$	Vinyldimethyl(acetoxymethyl)silane
3	10.609	7.08	$C_{15}H_{24}$	Caryophyllene
4	11.106	0.96	$C_{15}H_{24}$	.alpha.-Caryophyllene
5	11.255	17.70	$C_{12}H_{14}O_2$	2H-1-Benzopyran, 7-methoxy-2,2-dimethyl-
6	12.068	2.16	$C_{15}H_{24}$	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]-
7	12.995	1.55	$C_{16}H_{34}$	Hexadecane
8	13.166	1.12	$C_{13}H_{14}O_3$	Benzoic acid, 3-(3-hydroxy-3-methyl-1-butynyl)-
9	14.059	3.08	$C_{14}H_{16}O_2$	3-Isobutylidene-6,7-dimethyl-3H-isobenzofuran-1- one
10	14.282	1.90	$C_{10}H_{16}O_2$	2H-Pyran-2-one, 5,6-dihydro-6-pentyl-
11	14.580	24.87	$C_{15}H_{11}N_3$	6-Methyl-benzo [4,5] imidazo[1,2-c] quinazoline
12	15.364	1.60	$C_{18}H_{38}$	Octadecane
13	15.461	1.00	$C_8H_{14}O$	Cyclohexanemethanol, 4-methylene-
14	15.810	1.73	$C_{10}H_{18}$	Bicyclo [3.1.1] heptane,2,6,6-trimethyl-, [1S-(1. alpha.,2. beta.)]-
15	16.737	1.50	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
16	17.263	6.52	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
17	17.486	1.32	$C_{20}H_{42}$	Eicosane
18	18.511	1.18	$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)-, methyl ester
19	18.654	6.63	$C_{20}H_{40}O$	Phytol
20	19.014	6.06	$C_{18}H_{32}O$	9,17-Octadecadienal, (Z)-
21	19.186	1.55	$C_{18}H_{34}O_2$	cis-Vaccenic acid
22	19.421	1.16	$C_{18}H_{38}$	Octadecane
23	19.901	1.09	$C_{11}H_{16}N_2$	4,4,5,5-Tetramethyl-4,5-dihydro-1H-azepine-2-carbonitrile
24	29.806	4.01	$C_{24}H_{38}$,$C_{30}H_{50}$	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all E)-

Table 4.
Results of LD_{50} test of methanol extract of *Parquetina nigrescens* leaf

	No of animals used	**Dosage of MEPL**	**No of dead animals**	**No of life animals**
Step 1	3	2000 mg/kg	0	3
Step 2	3	2000 mg/kg	0	3

Table 5.
Effect of methanol extract of *Parquetina nigrescens* leaf on blood profile in arsenic trioxide-treated Wistar rats

	PCV (%)	**RBC (10^6/µL)**	**HB (g/Dl)**	**WBC (10^3/µL)**	**PLATELETS (10^3/µL)**
Group 1	38.60 ± 2.54	6.25 ± 0.45	12.62 ± 0.87	6.82 ± 1.09	123.00 ± 17.10
Group 2	35.00 ±2.02	5.73 ± 0.34	11.56 ± 0.73	4.37 ± 0.8*	148.00 ± 10.01
Group 3	35.20 ± 1.56	5.65 ± 0.25	11.36 ± 0.42	5.43 ± 0.66	187.00 ± 14.35*
Group 4	37.00 ± 1.89	6.11 ± 0.36	11.46 ± 0.65	5.10 ± 0.32	229.00 ± 25.72
Group 5	33.60 ± 0.24*	5.00 ± 0.02*	11.56 ± 0.12	6.50 ± 0.55	183.75 ± 21.80
Group 6	35.00 ± 0.44	5.55 ± 0.15	11.50 ± 0.20	6.67 ± 0.40$^+$	201.80 ± 50.63^{*+}
Group 7	34.80 ± 0.73	5.70 ± 0.22	11.66 ± 0.29	6.42 ± 0.78$^+$	185.20 ± 31.52^{*+}
Group 8	36.20 ± 0.73	5.73 ± 0.22	11. 80 ± 0.19	6.10 ± 0.23	136.00 ± 31.63

Values represent mean ± SEM, n = 5. $^{*,+}p < 0.05$ as compared with Control and As_2O_3 respectively.
Group 1= Control; Group 2= As_2O_3; Group 3= 250 mg/kg MEPL; Group 4= 500 mg/kg MEPL; Group 5= 1000 mg/kg MEPL; Group 6= 250 mg/kg MEPL + As_2O_3; Group 7= 500 mg/kg MEPL + As_2O_3; Group 8= 1000 mg/kg MEPL + As_2O_3

***In vitro* antioxidant assay**

***In vitro* metal chelating activity of methanol extract of *Parquetina nigrescens* leaf:** The IC_{50} of MEPL needed to chelate metal was significantly ($p < 0.05$) higher than that required of the standard (EDTA) to perform the same task (Table 2).

2,2-diphenyl-1-picrylhydrazyl scavenging activity of methanol extract of *Parquetina nigrescens* leaf: The IC50 of MEPL required to scavenged 2,2-diphenyl-1-picrylhydrazyl was significantly ($p < 0.05$) higher than that of the standard (vitamin C) (Table 2).

2,2'-azino-bis(3- ethylbenzothiazoline-6-sulfonic acid) scavenging ability of methanol extract of *Parquetina nigrescens* leaf : It was observed that IC50 of MEPL required to scavenge 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) was significantly ($p < 0.05$) higher when compared to standard (trolox) (Table 2).

Flavonoid content in methanol extract of *Parquetina nigrescens* leaf: Flavonoid concentration in MEPL was significantly ($p < 0.05$) higher when compared to standard (quercetin) (Table 2).

Quantity of phenol in methanol extract of *Parquetina nigrescens* leaf: The phenol content of MEPL was significantly ($p < 0.05$) lower when compared to standard (gallic acid) (Table 2).

Chemical compounds present in methanol extract of *Parquetina nigrescens* leaf: Characterisation as well as identification of chemical compounds in MEPL were carried out Table 3 showed the retention time (RT) which represents the relative concentration of compounds being eluted, the peak height (area%) which represents the relative concentrations of compounds present in the extract, the chemical formula as well as the library/ID of the 24 essential oils present in MEPL.

LD_{50} determination of methanol extract of *Parquetina nigrescens leaf*: The lethal dose (LD_{50}) of MEPL was greater than 5,000 mg/kg body weight (Table 4).

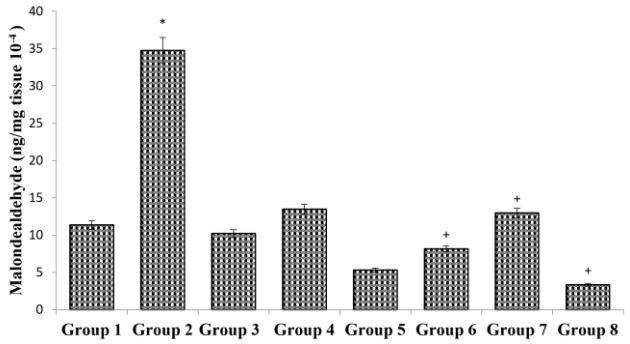

Figure 1.
Effect of MEPL on liver malondialdehyde concentration in arsenic trioxide-treated rats. *Columns represent mean ± SEM, n = 5. *, +p < 0.05 as compared with Control and As_2O_3 respectively.*
Group 1= Control; Group 2= As_2O_3; Group 3= 250 mg/kg MEPL; Group 4= 500 mg/kg MEPL; Group 5= 1000 mg/kg MEPL; Group 6= 250 mg/kg MEPL + As_2O_3; Group 7= 500 mg/kg MEPL + As_2O_3; Group 8= 1000 mg/kg MEPL + As_2O_3.

Effect of methanol extract of *Parquetina nigrescens* leaf on blood profile in arsenic trioxide-treated Wistar rats: The PCV and RBC count reduced ($p < 0.05$) significantly in the group treated with 1000 mg/kg MEPL when compared with the group (Table 5). There was no significant difference in haemoglobin concentration in all experimental groups when compared with the control group (Table 5). The white blood cell count reduced ($p < 0.05$) in As_2O_3 only group when compared with the control group. Also, an increase ($p < 0.05$) in WBC count was observed in the groups administered with As_2O_3 + 250 mg/kg, As_2O_3 + 500 mg/kg and As_2O_3 +1000 mg/kg MEPL when compared to As_2O_3 alone group (Table 5). Platelet count increased ($p < 0.05$) in 250 mg/kg MEPL group when compared to control group. Also, an increase in platelet count was observed in As_2O_3 + 250 mg/kg MEPL when compared to control group and with As_2O_3 only group (Table 5).

Effect of methanol extract of *Parquetina nigrescens* leaf on liver malondialdehyde concentration in arsenic trioxide-treated Wistar rats: Malondialdehyde concentration increased ($p < 0.05$) in As_2O_3 group when compared to control group. There was also a significant decrease ($p < 0.05$) in MDA concentration of groups administered with As_2O_3 + 250 mg/kg MEPL, As_2O_3 + 500 mg/kg MEPL and As_2O_3 + 1000 mg/kg MEPL when compared with group treated with As_2O_3 only (Figure 1).

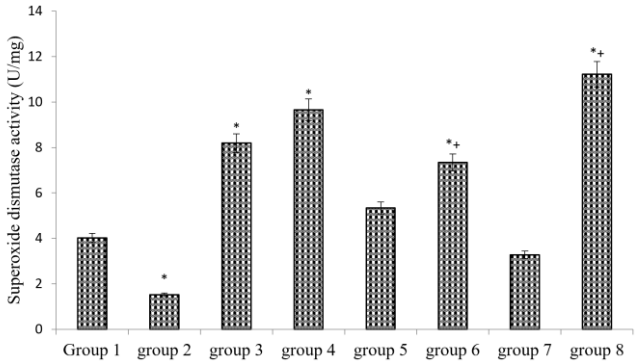

Figure 2.
Effect of MEPL on liver superoxide dismutase activity of arsenic trioxide-treated Wistar rats.
*Columns represent mean ± SEM, n = 5. *, +p < 0.05 as compared with Control and As_2O_3 respectively.*
Group 1= Control; Group 2= As_2O_3; Group 3= 250 mg/kg MEPL; Group 4= 500 mg/kg MEPL; Group 5= 1000 mg/kg MEPL; Group 6= 250 mg/kg MEPL + As_2O_3; Group 7= 500 mg/kg MEPL + As_2O_3; Group 8= 1000 mg/kg MEPL + As_2O_3.

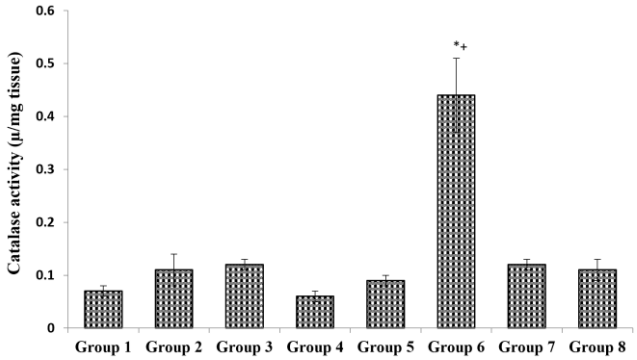

Figure 3.
Effect of MEPL on liver catalase activity in arsenic trioxide treated rats.
*Columns represent mean ± SEM, n = 5. *, +p < 0.05 as compared with Control and As_2O_3 respectively.*
Group 1= Control; Group 2= As_2O_3; Group 3= 250 mg/kg MEPL; Group 4= 500 mg/kg MEPL; Group 5= 1000 mg/kg MEPL; Group 6= 250 mg/kg MEPL + As_2O_3; Group 7= 500 mg/kg MEPL + As_2O_3; Group 8= 1000 mg/kg MEPL + As_2O_3

Effects of methanol extract of *Parquetina nigrescens* leaf on liver superoxide dismutase activity in arsenic trioxide treated Wistar rats: Superoxide dismutase activity decreased significantly in As_2O_3 group while an increase

was observed in 250 mg/kg MEPL as well as 500 mg/kg MEPL ($p < 0.05$) groups compared to control. The groups administered with As_2O_3 + 250 mg/kg MEPL and As_2O_3 + 1000 mg/kg MEPL showed an increase ($p < 0.05$) compared with As_2O_3 only and control groups respectively (Figure 2).

Effect of methanol extract of *Parquetina nigrescens* leaf on liver catalase activity in arsenic trioxide-treated Wistar rats: An increase ($p < 0.05$) in liver catalase activity was observed in As_2O_3 + 250 mg/kg MEPL when compared to control group and As_2O_3 only group (Figure 3).

Effect of methanol extract of *Parquetina nigrescens* leaf on liver aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities in arsenic trioxide-treated Wistar rats: There was an observed increase ($p < 0.05$) in liver AST in As_2O_3 group and in 500 mg/kg MEPL group in relations to control group. Also, AST decreased ($p < 0.05$) in groups administered As_2O_3 + 500 mg/kg MEPL or As_2O_3 +1000 mg/kg MEPL when compared to As_2O_3 group (Table 6).

Liver Alanine Aminotransferase (ALT) activity increased ($p < 0.05$) in As_2O_3 only group when compared with control group while, a decrease ($p < 0.05$) was observed in the groups administered As_2O_3 + 500 mg/kg MEPL and As_2O_3 +1000 mg/kg MEPL when compared to As_2O_3 only group (Table 6).

Alkaline Phosphatase (ALP) activity increased ($p < 0.05$) significantly in As_2O_3 group and in 250 mg/kg MEPL when compared with control group. Also, an increase ($p < 0.05$) was observed in groups treated with As_2O_3 + 250 mg/kg MEPL, As_2O_3 + 500 mg/kg MEPL and As_2O_3 + 1000 mg/kg MEPL ($p < 0.05$) when compared to control group (Table 6).

Table 6.
Effect of methanol extract of *Parquetina nigrescens* leaf on liver enzymes in arsenic trioxide-treated Wistar rats

	AST (U/I)	ALT (U/I)	ALP (IU/L)
Group 1	30.26 ± 1.14	33.93 ± 5.04	17.76 ± 5.68
Group 2	46.68 ± 1.08*	53.42 ± 12.38*	54.49 ± 6.11*
Group 3	29.55 ± 4.77	25.68 ± 7.27	34.14 ± 5.84
Group 4	52.63 ± 2.36*	22.67 ± 5.92	35.17 ± 7.42*
Group 5	40.97 ± 0.06	36.52 ± 5.61	27.41 ± 4.65
Group 6	40.04 ± 5.55	37.59 ± 3.12	27.41 ± 5.70+
Group 7	32.77 ± 6.04+	26.88 ± 3.41+	32.59 ±4.09+
Group 8	19.07 ± 2.62*+	30.06 ± 5.38+	55.00 ± 4.20*

*Values represent mean ± SEM, n = 5. *+p < 0.05 as compared with Control and As_2O_3 respectively.*
Group 1= Control; Group 2= As_2O_3; Group 3= 250 mg/kg MEPL; Group 4= 500 mg/kg MEPL; Group 5= 1000 mg/kg MEPL; Group 6= 250 mg/kg MEPL + As_2O_3; Group 7= 500 mg/kg MEPL + As_2O_3; Group 8= 1000 mg/kg MEPL + As_2O_3

Liver histology: The liver histology of control group showed normal central venules and portal tract. There was mild infiltration of the sinusoids by inflammatory cells. The hepatocytes appeared normal. The histology of the arsenic trioxide group showed normal central venules but mildly congested portal tract, mild infiltration by inflammatory cells was observed in the sinusoids and some of the hepatocytes had hypochromatic nuclei and vacuolated cytoplasm. The groups administered with 250 mg/kg MEPL, 500 mg/kg MEPL, As_2O_3 + 250 mg/kg MEPL and As_2O_3 + 500 mg/kg showed normal central venules and portal tract and normal hepatocytes, and no infiltration was seen in the sinusoids. The 1000 mg/kg MEPL is comparable to that of control group. The group treated with As_2O_3 + 1000 mg/kg MEPL showed mild vascular congestion, but the sinusoids and the hepatocytes appeared normal (Plate 1).

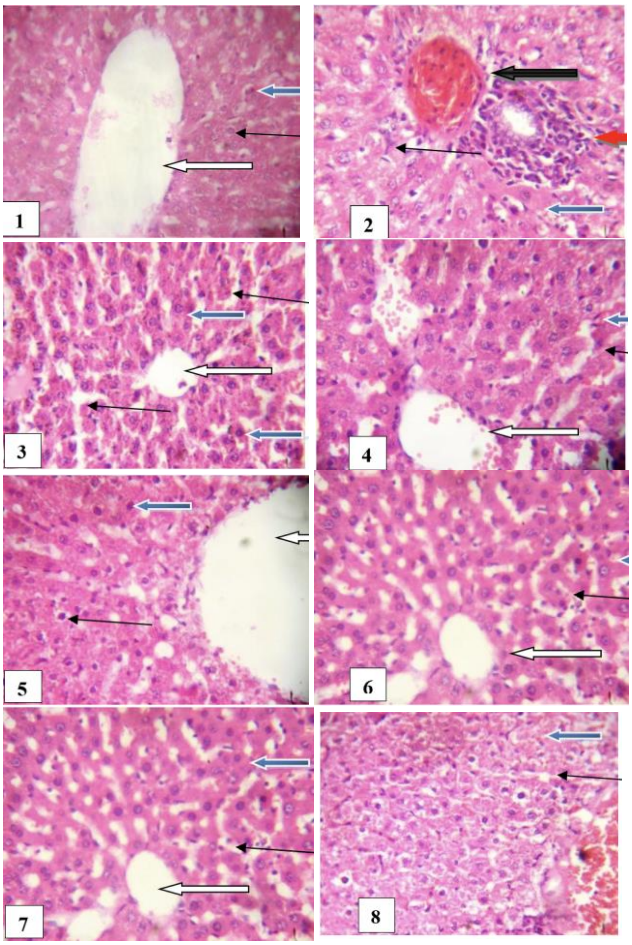

Plate 1: Liver histology stained with H&E (x400). (1) Control (2) As_2O_3 (3) 250 mg/kg MEPL (4) 500 mg/kg MEPL (5) 1000 mg/kg MEPL (6) 250 mg/kg MEPL + As_2O_3 (7) 500 mg/kg MEPL + As_2O_3 (8) 1000 mg/kg MEPL + As_2O_3. White arrow indicates central venules, blue arrow indicates normal morphology of hepatocytes, black arrow indicates mild congestion of portal vein, red arrow indicate mild periportal infiltration and black arrow indicates the sinusoids.

DISCUSSION

The presence of alkaloids, tannins, saponins, flavonoids as well as anthraquinnones in MEPL are consistent with the report of Ayoola *et al*. (2011). The presence of flavonoid in MEPL as revealed by phytochemical screening was also confirmed by the result of the *in vitro* antioxidant assay in which the flavonoid content of MEPL surpassed that of standard quercetin. Flavonoids are antioxidants and anti-inflammatory substances which prevent oxidative stress (Verma *et al*., 1976). Tannins were also found to be present in MEPL. Tannins are of two types, the first is the condensed and the second type is the hydrolysable which are composed of monosaccharide core. The latter is less stable and has the potential to cause toxicity (Bernhoft, 2010). Tannins are used as astringents in case of diarrhoea and can also hasten wound healing. They ameliorate inflamed mucus membrane (Leal *et al*., 2015). Tannin has been experimentally proven not to adversely affect the liver but rather protect it from toxic effects of insults such as carbon tetrachloride (Chu *et al*., 2016; Elfadil *et al*., 2013).

Saponin was also found to be one of the phytochemical components of MEPL. Saponin possesses anti-neoplastic influence

and immune modulatory influence (Bernhoft, 2010). Saponin has been credited with the ability to protect liver from acetaminophen-induced liver damage as well as from water immersion-stress induced liver damage both in mice (Kim *et al*., 2009; Zhao *et al*., 2012). Saponin has also been reported to elicit a negative effect on erythrocytes by causing cell shrinkage and membrane scrambling of erythrocytes (Bissinger *et al*., 2014)

Alkaloids are of different types, examples are; pyrrolizidin alkaloids, tropane alkaloids, methylxantine alkaloids, etc. Experiment has shown that plant derived alkaloids can stimulate humoral and mediated immunity by stimulating WBC as well as platelets production in the bone marrow (Bachhav and Sambathkumar, 2016).

Anthraquinones are described to have several biological effects such as antioxidant, anti-inflammatory, antibacterial, diuretic, antitumor, antiathritic, antifungal, antibacterial and antimalarial activities and can thus protects from a wide range of diseases (Raji *et al*., 2005; Dave and Ledwani, 2012 Chen *et al*., 2015).

It was observed that MEPL had metal chelating effect. Although, the quantity of MEPL required to achieve metal chelating effect was high with respect to the standard (ethylenediamine tetraacetic acid), yet it was glaring that MEPL had chelating property. Metal chelators help to prevent heavy metal-induced toxicity in the body by chelating them and make them available for excretion (Flora *et al*., 2007). Thus, MEPL may likely chelate heavy metals and therefore prevent oxidative stress.

Diphenyl-1-picrylhydrazyl, (DPPH) is a stable free radical, DPPH assay provides opportunity of evaluating the potential of a substance to function as a scavenger or as H^+ donor (Kedare and Singh, 2011). The quantity of MEPL needed to scavenge free radicals when measured against standard was found to be higher, but notwithstanding, MEPL demonstrated the capacity to scavenge DPPH. This observation corresponds with that of Ayoola *et al.* (2011) where the scavenging activities of flavonoid extract and methanol extract of *Parquetina nigrescens* leaf exhibited a higher scavenging power against butylated hydroxyanisole.

2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) ($ABTS^+$) upon introduction of potassium persulphate becomes a radical cation and like the DPPH free radical, $ABTS^+$ is employed in the confirmation of claimed or proposed antioxidant capacity of extracts (Re *et al*., 1999). This study showed that MEPL elicited free radical scavenging ability against $ABTS^+$. Although the quantity (IC_{50}) of MEPL that scavenged $ABTS^+$ was more than the quantity (IC_{50}) of standard (trolox) that did similar work. However, this study has established that MEPL has a scavenging ability against $ABTS^+$ and may thus be reckoned with as an antioxidant agent.

The reason for increased concentration of MEPL to chelate metal and scavenge free radical may not be far-fetched as the standard (trolox or quercetin) is a pure compound that can carry out its function with minimal interference from impurities while MEPL is composed of 24 essential oils and a host of other phytochemical as revealed in this study and its active components against metals, $ABTS^+$ or DPPH may likely be interfaced with other components that are not needed to achieve the desired result and thereby, influence the measure of MEPL required to chelate metals or scavenge free radicals.

This finding corroborates the phytochemical screening in this study which revealed the presence of flavonoids in MEPL. Ayoola *et al*. (2011) reported that flavonoid extracted from MEPL demonstrated greater scavenging properties against DPPH than MEPL itself.

The *in vitro* antioxidant assay in this study revealed the presence of phenol in MEPL. Although, the phenol content of the standard (gallic acid) was more than that of MEPL. Plants that belong to the same family with *Parquetina nigrescens* have shown high content of phenol. Example of such is *Mondia whitei* which reportedly has very high polyphenol content (Bouba *et al*., 2010). Phenols are beneficial to health in several ways which includes eliminating reactive oxygene species, defending and regenerating other antioxidants derived from diet as well as chelating pro-oxidant metals. It also suppresses cell death and decreases markers of inflammation (Cicerale *et al*., 2009). The presence of phenol in MEPL may therefore strengthen plasma antioxidant capacities against free radicals.

The characterization study carried out showed 24 essential oils in MEPL, however, the ones present in significant amount were; Caryophyllene, 2H-1-Benzopyran, 7-methoxy-2,2-dimethyl-, 6-Methylbenzo[4,5]imidazo[1,2-c]quinazoline, n-Hexadecanoic acid, Phytol, 9,17-Octadecadienal, (Z)-, 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all E)-(squalene).

Caryophyllene also known as beta caryophyllene (BCP) is present in MEPL as established by the characterization study. It is also present in numerous herbs employed for various reasons (Calleja *et al*, 2013). It was approved by US FDA as additive and flavour in food because of its spicy odour and low toxicity (Adams *et al*., 2011). Experiments have shown that oral administration of BCP reduced pain caused by high temperature, elicited neuroprotection, anti-inflammatory and cytoprotective as well as antioxidant effects in rat (Fernandes *et al*., 2007; Calleja *et al*., 2013; Klauke *et al*., 2014; Hayate *et al*., 2016).

The 2H-1-Benzopyran, 7-methoxy-2,2-dimethyl- is also known as Precocene 1 (Halpin *et al*., 1984), It has been implicated in hepatic damage causing a time and dose dependent depletion of liver glutathione (GSH) activity (Halpin *et al*., 1984). It was also discovered that Precocene 1 had an insecticidal effect on *Coptotermes formosanus* and *Locusta migratory* (Mao *et al*., 2010).

The n-Hexadecanoic acid is a fatty acid otherwise called palmitic acid. Palmitic acid has been implicated in hypothalamic insulin resistance in rats by altering the central nervous control of insulin secretion and by suppressing signals emanating from insulin and leptin meant to inhibit appetite (Benoit *et al*., 2009). It was also reported to boost metastasis of human oral tumor cell (Pascual *et al*., 2017).

Phytol is a by-product of chlorophyll metabolism (McGinty *et al*., 2010). It is a precursor for producing synthetic forms of Vitamins E and K (Thomas, 2007). Studies have shown that phytol has anti-nociceptive, antioxidant, anti-inflammatory, antibacterial, anti-carcinogenic and anticonvulsant effects (Saikia *et al*., 2010; Costa *et al*., 2012; Santos *et al*., 2013; Silva *et al*., 2014; Song and Cho, 2015).

Squalene is a triterpene, it is traditionally used in wound healing and for curing various respiratory tract conditions (Tijan, 2001). It plays an essential role in the synthesis of cholesterol (Spanova and Dum, 2011). It is not exactly vulnerable to peroxidation and it is stable against the attack of peroxide radical (Kim *et al*., 2003). Squalene has been reported to ameliorate hypercholesterolemia and prevent coronary heart disease, it has also been suggested to possess anticancer, cytoprotective and androgenic activities in rats (Liu *et al*., 2009; Popa *et al*., 2014).

The chromatogram with the highest percentage, 6-Methyl-benzo[4,5]imidazo[1,2-c]quinazoline (24.87%) has got only a handful information on it, however, its derivatives have been reported to possess antibacterial effects against gram negative bacteria and also antifungal activity (Nandwana *et al*., 2018).

In this study, the LD_{50} of MEPL was greater than 5000 mg/kg body weight using limit test in OECD 423 (2001). Aderibigbe *et al*. (2011) used Miller and Tainter's method (1944) and reported that LD_{50} of MEPL was 4500 mg/kg. Also, Awobajo *et al*. (2010) reported the LD_{50} of aqueous extract of *Parquetina nigrescens* to be 12.60 g/kg and 13.10 g/kg using Thompson (1947) and Finney (1952) methods. Such wide variations in result is possible because water cannot extract the non-polar substances like methanol (Ngo *et al*., 2016), thereby making the methanol extract to contain more components which may of course raise the toxicity level. This result indicates that at a high dosage, the consumption of MEPL is safe and this informed the choice of the dosages used in this study.

It was observed that 1000 mg/kg of MEPL brought about a reduction in packed cell (PCV) volume as well as red blood cell (RBC) count. This contradicts Saba *et al*. (2010) where treatment with 1000 mg/kg aqueous extract of *Parquetina nigrescens* for four weeks did not cause any change in both PCV and RBC. Also, the result of this study contradicts the reports of Agbor and Odetola (2001) and Omoboyowa *et al*. (2016) in which administration of *Parquetina nigrescens* caused marked increase in both PCV and RBC. The variance of this study from previous studies could be due to difference in dosage or length of period of administration. *In-vitro* exposure to tannins and saponins which are also present in *Parquetina nigrescens* were reported to cause hemolysis and eryptosis of RBC (Bissinger *et al*., 2014). It is therefore possible that the long-time exposure of the experimental animals to MEPL at a high dosage of 1000 mg/kg caused an increased bioavailability of saponin and tannin in the extracellular fluid which may have had an adverse effect on the PCV and RBC count.

In this study, it was observed that there was a decreased WBC count in 3 mg/kg of arsenic only. This agrees with Kannan *et al*. (2001). Previous work done by other researchers revealed that exposure of experimental animals to arsenic causes leucopenia (Kyle and Pearse, 1965). The mechanism through which arsenic cause decreased WBC count may be through its capacity to cause apoptosis (Rousselot *et al*., 1999). However, it was observed that the effects of arsenic on WBC count was ameliorated by 250, 500 and 1000 mg/kg MEPL. Since increase in WBC count was not observed in MEPL only treated groups, but in the groups co-treated with arsenic and MEPL, the increase may likely be based on stimulation of granulopoietin in reaction to the reduced cells respectively (Saba *et al*., 2010). The phytochemical screening of MEPL showed the presence of alkaloids. Alkaloid fraction from other plants have been reported to stimulate bone marrow activity by increasing the production of WBC and platelets (Bachhav and Sambathkumar, 2016).

The observed increase in platelet count in the groups administered 250 mg/kg MEPL and As_2O_3 + 250 mg/kg MEPL might have been triggered by the consistent administration of MEPL for a period of 54 days. This showed that MEPL may facilitate thrombocytosis and may bring about quick haemostatic response to blood vessel damage. The normal range of value for platelet count in rat was given as 150 – 460 x 10^3/mL by Johnson-Delaney (2008) and this shows that the increment in platelet count in this experiment was not detrimental to the health of the animals In this study, a rise in liver MDA concentration was seen in 3 mg/kg arsenic group only. This conforms to the report of Abu El-Saad *et al*. (2016) and Klibet *et al*. (2016). The measurement of MDA concentration is a means of evaluating lipid peroxidation level caused by oxidative stress. This result suggests a marked increase in free radicals in the liver which might have been mediated by arsenic treatment. It was however observed that all three dosages of MEPL ameliorated the lipid peroxidation effect of arsenic trioxide in the liver. The phytochemical constituents of *Parquetina nigrescens* leaf as revealed in this study showed the presence of phenol and flavonoids that participate in scavenging of free radicals. These phytochemicals might have mopped off the lipid peroxidative effects of arsenic in the liver cells which eventually lead to decreased MDA concentration in arsenic groups treated with all the three doses of MEPL.

Arsenic trioxide caused a decrease in liver SOD activity of arsenic only treated group. Arsenic generates large number of free radicals which overwhelms the antioxidant defence mechanism and thus alters SOD expression (El-Demerdash *et al*., 2009). It was however observed that SOD activity improved in the groups treated with arsenic and co-administered with 250 or 1000 mg/kg MEPL. This agrees with Ayoola *et al*. (2011) who suggested that flavonoid extract of MEPL induced production of SOD. This statement could be viewed as true because, the administration of 250 and 500 mg/kg MEPL alone brought about an increase in SOD activity in this study.

The catalase activity was high only in arsenic group co-administered 250 mg/kg MEPL, the presence of arsenic could have led to the expression of catalase in liver cells in copious amount to keep the LPO activity of arsenic under check.

The liver carries out an important function in metabolism as every ingested substance is subjected to first pass effect in the liver, it was therefore deemed fit to evaluate the influence of both arsenic and MEPL administration on liver. The AST, ALT and ALP are produced by liver in large amount; they serve as indicator of hepatic cell injury (Fattovich *et al*., 2008). In this study, increase in the three enzymes was seen in the arsenic trioxide only treated group. This agrees with Messarah *et al*. (2012). The decrease in AST and ALT in the groups co-administered with arsenic and 500 or 1000 mg/kg MEPL suggests that it has hepatoprotective effect. The ALP concentration was attenuated in As_2O_3 + 250 mg/kg MEPL and As_2O_3 + 500 mg/kg but not in As_2O_3 + 1000 mg/kg. This could mean that MEPL is more effective against liver damage with the attending evidence of ALP at dosages lower than 1000 mg/kg

In this study, the liver histology of As_2O_3 only group showed mildly congested portal tract, mild infiltration of the sinusoids by inflammatory cells, some of the hepatocytes had hypochromatic nuclei and vacuolated cytoplasm. This agrees with the works of Zhang *et al*. (2014). Liver function is usually assessed by assaying for the level of AST, ALT and ALP in the blood (Fattovich *et al*., 2008) and a high concentration of these three enzymes indicates degeneration of liver hepatocytes. The abnormal liver histology in As_2O_3 only group was in line with the high concentrations of AST, ALT and MDA that were obtained in this study and thus, establishes the hepatotoxic effects of arsenic trioxide. The liver of the groups treated with As_2O_3 + 250 mg/kg, As_2O_3 + 500 mg/kg and As_2O_3 + 1000 mg/kg showed improved histology which may be credited to MEPL. The result of liver histology, liver redox status, antioxidants as well as liver enzymes in groups treated with arsenic trioxide and co-treated with different dosages of MEPL indicated that MEPL has hepatoprotective effect which may be attributed to its antioxidant capacity.

In conclusion, this study revealed that the phytochemical constituents of methanol extract of *Parquetina nigrescens* leaf, has potent antioxidant abilities as well as metal chelating capacity. The high LD_{50} of MEPL shows a high safety margin for its consumption. Furthermore, MEPL improved WBC count and protected liver cells from arsenic trioxide-induced toxicity as was evidenced by its ability to decrease MDA, ALT and AST while it improved SOD, catalase as well as the histology of liver tissue. Thus, methanol extract of *Parquetina nigrescens* leaf has antioxidant effects that may be explored on the various physiological systems.

Acknowledgement

The authors would like to acknowledge and appreciate the members of Reproductive Physiology and Developmental Programming unit, Department of Physiology, University of Ibadan for their kind assistance and support toward this study.

REFERENCES

Abu El-Saad, A. M., Al-Kahtani, M. A. and Abdel-Moneim, A. M. (2016). N-acetylcysteine and meso-2,3 dimercaptosuccinic acid alleviate oxidative stress and hepatic dysfunction induced by sodium arsenite in male rats Dovepress 2016 (10): 3425-3434.

Adams, T. B, Gavin, C. L, Waddell, W. J, McGowen, M. M, Cohen, S. M. and Feron, V. J. (2011). The FEMA GRAS assessment of aliphatic and aromatic terpene hydrocarbons used as flavor ingredients. Food Chem. Toxicol. 49: 2471 - 2494.

Aderibigbe, O. R., Odetola, A. A., Oluwole, F. S., Farombi, E. O., Onabanjo, O. O. and Jiboku, O. A. (2011). Antioxidant properties of methanol extract of *Parquentina nigrescens* in ulcerated rats. Int. J. Trop. Med. 6 (2): 25-29.

Afolabi, O. M., Ajayi, I. R., Olubosede, O, Fayose, R. S. and Sunday, A. G. (2011). Arsenic, Nickel and Iron Concentration levels in Water Samples from Hand-Dug Wells from Ugbe Akoko. Am. J. Appl. Sci. 8 (2): 182 - 185.

Agbor, A. G. and Odetola, A. A., (2001). Hematological studies of *Parquetina nigrescens* on haemorrhagic anaemic rats. Afri. J. Med. Med. Sci. 30: 105 – 109.

Ahoule, D. G. A., Lalanne, F., Brosillon, s, Mendret, J. and Maiga, A. H. (2015). Arsenic in African waters: A review. Water air soil pollut. 226: 302.

Alvarez, C. N. S. (2012). *Parquetina nigrescens* (Afzel.) Bullock. In: Schmelzer, G.H. & Gurib-Fakim, A. (Eds). Medicinal plants/Plantes médicinales. Wageningen, Netherlands, Prota 11: (2).

ATSDR. (2007). Toxicological Profile for Arsenic. Agency for Toxic Substances and Disease Registry, U.S. Department of Publics Health and Human Services, Atlanta, GA.

Awobajo F. O., Omorodion-Osagie E., Olatunji-Bello, I. I., Adegoke, O. A. and

Adeleke T. I. (2010). Acute oral toxicity and phytochemistry of some West African Medicinal Plants. Nig. Q. J. Hosp. Med. 19(1): 53 - 8.

Ayoola, A. O., Akinloye, O., Oke, J.M., Oguntibeju, O. O. and Odetola, A. A. (2011). Antioxidant activities properties of methanol extract of Parquentina nigrescens in ulcerated rats; Afri. J. Biotechnol. 10 (24): 4920 - 4925.

Bachhav, R. S. and Sambathkumar, R. (2016). Evaluation of immunomodulatory activity of the alkaloid fraction of Trichopus Zeylanicus gaertn on experimental animals. Indian J. Pharm. Sci. 78 (1): 161 - 166.

Benoit, S. C., Kemp, C. J., Abplanalp, W., Elias, C. F., Herman, J. P, Lefevre, A. L., Migrenne, S, Cruciani-Gulglielacci C., Magan C, Yu F., Niswender K, Irani B. G., Holland W. l. and Clegg D. J. (2009). Palmitic acid mediates hypothalamic insulin resistance by altering PKC-Ɵ subcellular localization in rodents. J. Clin. Investig. 119 (9): 2577 - 2587.

Bergquist, E. R., Sugden, K. D, Fischer, R. J. and Martin, B. D. (2009). Inhibition by methylated organo-arsenicals of the respiratory 2-oxo-acid dehydrogenases. J. Organomet Chem. 694: 973 – 980.

Bernhoft, A. (2010). A Brief Review on Bioactive Compounds in Plants. In: Bernhoft, A. (Ed). Bioactive Compounds in Plants: Benefits and Risks for Man and Animals. Novus Forlag, Oslo, Norway, pp: 11 - 18.

Bhowmik, D., Chiranjib, K. P. and Kumar, S. 2010. A potential medicinal importance of zinc in human health and chronic disease. Int. J. Pharm. Biomed. Sci. (1): 5-11.

Bissinger, R., Alzoubi, K., Modicano, P., Honisch, S., Faggio, C., Abed, M. and Lang F. (2014). Effect of saponin on erythrocytes. Int. J. Hematol. 100: 51 – 59.

Blois, M.S. (1958). Antioxidant Determinations by the Use of a Stable Free Radical. Nature 181: 1199 - 1200.

Bouba, A., Scher, J, Njintang, Y. N, and Mbofung, C. M. F. (2010). Phenolic compounds and radical scavenging potential of twenty Cameroonian spices. Agric. Biol. J. N. Am. 1.3: 213 - 224.

Bradl, H. (Ed). (2002). Heavy metals in the environment: Origin, interaction and remediation. Elsevier/Academic press, London, pp 269.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. 28: 25 – 30.

Calleja, M. A., Montero-Meterdez, T., Vieites, J. M., Faus, M. J., Torres, M. I., Gil, A. and Suárez, A. (2013). The antioxidant effect of β-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. Br. J. Nutr. 109: 394 – 401.

Chen, S. C., Wu, Y. C., Chen, Z. W. and Yang, W. C. (2015). Naturally Occurring Anthraquinones: Chemistry and therapeutic potential in autoimmune diabetes. Evid. -based Complement. Altern. Med. 2015:1 - 13.

Chu, X.,Wang, H., Jiang, Y., Zhang, Y., Bao, Y., Zhang, X., Zhang, J., Guo, H., Yang, F., Luan, Y. and Dong, Y. (2016). Ameliorative effects of tannic acid on carbon tetrachloride-induced liver fibrosis in vivo and in vitro. J. Pharmacol. Sci. 130(1): 15 - 23.

Cicerale, S, Sinclair, A. J, Conlan, X. A. and Keast, R. S. J. (2009). Chemistry and health of olive oil phenolics. Crit. Rev. Food Sci. Nutr. 49: 218 – 236.

Cohen, S. M., Eldan, M., Lewis, A. S., Arnold, L. L. and Beck, B. D. (2006). Methylated arsenicals: the implications of metabolism and carcinogenicity studies in rodents to human risk assessment. Cri. Rev. of Toxicol. 36: 99 – 133.

Constantin, M. and Alexandru. I. 2011. The role of sodium in the body. Balneo PRM J 2(2): 69-74.

Costa, J. P., De Sousa, Ferreira, P. B., D. P., Jordan, J., Freitas, R. M. (2012). Anticonvulsant effect of phytol in pilocarpine model in mice. Neurosci. Lett. 523.2: 115 - 118.

Cullen, W. R. and Reimer, K. J. 1989. Arsenic speciation in the environment. Chem. Rev. 89 4: 713 – 764.

Datte, J., Offoumou, A. M., and Manda, O. M. (1996). Uterotonic effects of hydromethanol extract of *Parquetina nigrescens* (Periplocaceae) on spontaneous contractile activity in the isolated myometrium of pregnant rats. J. Ethnopharmacol. 53: 15 – 20.

Dave, H. and Ledwani, L. (2012). A review on anthraquinones isolated from Cassia species and their applications. Indian J. Nat. Prod. Resour. 3.3: 291 - 319.

Edeoga, H. O., Okwu, D., E. and Mbaebe, B. O. 2005. Phytochemical constituents of some Nigerian medical plants. Afr. J. of Biotechnol. 4.7: 685-688.

Egbinola, C. N and Amanambu A. C. (2014). Ground water contamination in Ibadan, South-West Nigeria. Springerplus 20(3): 448.

El-Demerdash, F. M., Yousef, M. I., Mokhtar, I. and Radwan, F. M. E. (2009). Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs. Food Chem. Toxicol. 47: 249 – 254.

Elfadil, A. G., Sabahelkhier, M. K., Rayan, M. Y., Daa, M. O., Nagla, A. H. and Israa, S. B. (2013). Effect of Tannin and Plant Tannins on some Organs and Physic-Chemical Characters of Diabetic Wistar Rats. Int. J. Adv. Res. 1 (10): 165 - 170.

Erah, P. O., Asonye, C. C. and Okhamafe, A. O. (2003). Response of Trypanosoma brucei brucei-induced anaemia to a commercial herbal preparation. Afr. J. Biotechnol. 2 (9): 307– 311.

Fattovich, G., Olivari, N., Pasino, M., D'Onofrio, M., Martone, E. and Donato, F. (2008). Long –term outcome of chronic hepatitis B in Caucasian patients: mortality after 25 years. Gastroenterol. 57: 84-90.

Fernandes, E. S., Medeiros, R., Passos, G. F., Da Cunha, F. M., Campos, M. M., Ferreira, J., Pianowski, L. F. and Calixto, J. B. (2007). Anti-inflammatory effects of compounds alpha-humulene and (-)-trans-caryophyllene isolated from the essential oil of cordial verbenacea. Eu. J. Pharmacol. 569(3): 228 - 36.

Finney, D, J. (1952). Probit analysis. J. Inst. Actuar. 78 (3): 388-390.

Flora, S. J. S., Bhadauria, S., Kannan, G. M. and Singh, N. (2007). Arsenic induced oxidative stress and the role of antioxidant supplementation during chelation: A review. J. Environ. Biol. 28 (2): 333 - 347.

Florea, A. M., Dopp, E., Obe, G. and Rettenmeier, A. W. (2004). Genotoxicity of organometallic species. In: Hirner, A. V., Emons, H. (Eds). Organic metals and metalloid species in the Environment: Analysis, Distribution, Processes, and toxicological evaluation. Springer-verlag, Heidelberg, pp 205 - 219.

Halpin, R. A., El-Naggar, S. F. Vyas, K. P. and Jerina, D. M. (1984). Metabolism and hepatotoxicity of the naturally occurring benzo[b]pyran precocene I. Chem. Biol. Interact. 48 (3): 297 - 315.

Hayate, J., Emadadul, H. M., Sheikh, A. and Shreesh, K. O. (2016). Cannabinoid type 2 (CB2) receptors activation protects against oxidative stress and neuroinflammation associated doperminergic neurodegeneration in Totenone model of Parkinson's disease. Front. Neurosci. 10: 321.

Iwu, M. M. 1993. Hand book of African Medicinal Plants. CRC press, Boca Raton, pp 464.

Johnson-Delaney, C. A. (2008). Exotic Animal Companion Medicine Handbook for Veterinarians. Zoological Education Network, Florida, pp 18/98.

Kannan, G., Tripathi, N., Shashi, D. N., Gupta, M and Flora S. (2001). Toxic effects of Arsenic (III) on some hematopoietic and central nervous system variables in rats and guinea pigs. J. Toxicol.Clin. Toxicol. 39 (7): 675 - 682.

Karasek, F. W. and Clement, R. E. (1988). Basic Gas Chromatography – Mass Spectrometry: Principles and Techniques, Elsevier Science Publishers Amsterdam, pp 201.

Kim, Y. J., Kim, T. W., Kwon, I. C., Chung, H., Sung, H. C. and Jeong, S.Y. (2003). The effects of serum on the stability and the transfection activity of the cationic lipid emulsion with various oils. Int. J. Pharmacol. 252: 241 - 252.

Kim, M. H., Lee, J., Yoo, D. S., Lee, Y. G., Byeon, S. E., Hong, E. K. and Cho, J. Y. (2009). Protective effects of stress-induced liver damage by saponin fraction from Codonopsis lanceolata. Arch. Pharm. Res. 32: 1441.

Klauke, A. L, Pradier, B, Racz, I, Zimmer. A. M., Markert, A. and Gertsch, J. (2014). The cannabinoid CB 2 receptor-selective phytocannabinoid beta-caryophyllene exerts analgesic effects in mouse models of inflammatory and neuropathic pain. Eur. Neuropsychopharmacol. l: 608 - 620.

Klibet, F., Khiari, M., Boumendjel, A., Abdennour., C. El Feki, A. and Messarah, M. (2016). Oxidative stress-related liver dysfunction by sodium arsenite: Alleviation by Pistacia lentiscus oil. Pharm. Biol. 54 (2): 354 - 363.

Kumar, A. R., Rathinam, K. M. and Prabhakar, G. (2007). Phytochemical screening of medicinal plants of Asclepiadaceae family. Asian J of Microbiol. Biotechnol. Envron. Sci. 1: 177-180

Kyle, R. A., and Pearse, G. L. (1965). Haematological aspects of arsenic intoxication. N. Engl. J. Med 273: 18 - 23.

Leal, A., Davi, D., Laynne, H. C. L., Nunes, L. C. C. and Jose A. D. L. (2015). Incorporation of tannic acid in formulations for topical use in wound healing: A technological prospecting. Afr. J. of Pharm. Pharmacol. 9 (26): 662-674.

Liu, Y., Xu, X., Bi, D., Wang, X., Zhang, X., Dai, H., Chen, S., Zhang. W. (2009). Influence of squalene feeding on plasma leptin, testosterone and blood pressure in rats. Indian J. Med. Res. 129 (2): 150-153.

Mao, L., Henderson, G. and Dong, S. L. (2010). Studies of precocene I, precocene II and biogenic amines on formosan subterranean termite (Isoptera: Rhinotermitidae) presoldier/soldier formation. J. Entomol. Sci. 45: 27-34.

McGinty, D., Letizia, C. S. and Api A. M. (2010). Fragrance material review on phytol. Food Chem. Toxicol. 48 (3): 59.

Messarah, M., Boumendjel, A., Klibet, F., Bouzerna, N., Abdennour, C., Salah, M., Boulakoud, M. S. and El Feki A. (2012). Hepatoprotective role and antioxidant capacity of selenium on arsenic-induced liver injury in rats. Exp. Toxicol. Pathol 64: 167–174.

Miliauskas, G., Venskutonis, P. R., & Van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 85: 231 – 237.

Miller L. C. and Tainter, M. L. (1944). Estimation of LD50 and its error by means of log-probit graph paper. Proc. Soc. Exp. Biol. Med. 57: 261.

Misra, H. P. and Fridovich, I. (1972). The generation of superoxide radical auto-oxidation of hemoglobin. J. Biol. Chem. 247: 6960 - 6962.

Nandwana, N. K., Singh, R. K., Patel, P. S., Dhiman, S, Saini, H. K., Jha, P. N and Kumar, A. (2018). Design and synthesis of Imidazo/Benzimidazo[1,2-c]quinazoline Derivatives and Evaluation of their antimicrobial activity. ACS Omega. 3(11): 16338 - 16346.

Ngo, T. V., Scarlett, C. J., Bowyer, M. C., Ngo, P.D. Vuong, Q. V. (2016). Impact of different extraction of solvents on bioactive compounds and antioxidant capacity from root of salacia chinensis L. J. Food Qual. 2017: 1 - 8.

NRC (National Research Council). (1999). Arsenic in drinking water. Washington, DC: National Academy Press.

Nriagu, J. O., and Azcue, J. M. (1990). Food contamination with arsenic in the environment. Adv. Environ. Sci. Technol. 23: 121-143.

Organization for Economic Development. (2001): OECD Guideline for the testing of chemical. Acute oral toxicity and acute toxicity class method. Test guideline 423: 1-14.

Omoboyowa, A. D., Igara, E. C. Ogunneye, L. A. and Otuchristian, G. (2016). Phytochemical screening and haematological studies of *Parquetina nigrescens* ethanol and chloroform leaf extracts in normal albino rats. Afri. J. Pharm. Pharmacog. 10.10: 164-169.

Parekh., J. and Chanda, S. V. 2007. Antimicrobial activity and phytochemical studies on twelve spicies of indian medicinal plants. Afri, J. Biomed. Res. 10: 175-181.

Pascual, G., Mejetta, S., Avgustinova, A., Castellanos, A., Martin, M., Attolini, C. S., Berenguer, A., Prats, N., Toll, A., Hueto, J. A., Bescos, C., Di Croce, L. and Benitah, S. A. (2017). Targeting metastasis-initiating cells through the fatty acid receptor CD36. Nature 541(7635): 41-45.

Popa, I., Niță, S., Băbeanu, N. E. and Popa, O. 2014. Squalene – Natural Resources and applications. Farmacia. 62 (5): 838-862.

Pravina, P., Sayaji, D. and Avinash, M. 2013. Calcium and its role in Human Body. Int. J Res. Pharm. Biomed. Sci. 4(2): 659-668.

Prieto, P., Pineda, M. and Aguilar M. 1999. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E1. Anal. Biochem. 269: 337–341.

Raji, y., Akinsomisoye, O. S., Salman, T. M. (2005). Antispermatogenic effect of Morinda Lucida Extract in male rats. Asian J. Androl. 7(4): 405-410.

Re, R., Proteggente, A., Pellegrini, N., Yang, M. Pannala, A. and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26: 1231–1237.

Reitman, S., Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Pathol. 28: 56-63.

Rice-Evans, C., Omorphos, C. S, Baysal, E. (1986). Sickle cell membrane and oxidative damage. Biochem. J. 237: 265-269.

Rousselot, P., Marolleau, J. P., Labaume, S., Noguera, M. H., Larghero, J. and Brouet, J. C. (1999). Arsenic trioxide and melarsoprol induce apoptosis in plasma cell lines and plasma cells from myeloma patients. Cancer Res. 59: 1041– 048.

Saba, A. B., Oyagbami, A. A., and Azeez O. L (2010). Antidiabetic and haematinic effect of Parquentina nigrescens on alloxan induced type-1 diabetes and normocytic normochromic anaemia in Wistar rats. Afri. Health Sci. 10 (3): 276-282

Saikia, D., Chanda, D., Parihar, S., Ojha, S. and Kumar, J. K. (2010). Antitubercular potential of some semisynthetic analogues of phytol. Bioorg. Med. Chem. Lett. 20: 508-512.

Santos, C. C. D.M. P., de Oliveira, G. A. L., Salvadori, M. S., de Sousa, D. P., Costa, J. P., Freita, R. M. and de Almeida, R. N. (2013). Antinociceptive and antioxidant activities of phytol in vivo and in vitro models. Neurosci. J. 2013: 1 - 9.

Scheindlin, S. 2005. The duplicitous nature of inorganic arsenic. Mol. Interv. 5: 60 –64.

Shittu, S. T., Oyeyemi, W. A., Shittu S. A. and Lasisi, T. J. (2018). Ucimum Gratissimum inhibits glycogen phosphorylase activity without changes in Hepatic nuclear factor Kappa B (NF-kB) and inducuble nitric oxide synthase (iNOS) in streptozotocin-induced diabetic rats. Niger. Med. Pract. 73 (1-2): 10 - 17.

Silva, R. O., Damasceno, S. R. B., Sousa, F. B. M., Carvalho, N. S., Oliveira, F. R. M. A., Sousa, D. P., Aragao, K. S., Silva, V. N. G., Barbosa, A. L. R., Freitas, R. M. and Medeiros J V. R. (2014). Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. Fundam. Clin. Pharmacol. 28: 455-464.

Singh, N. and Rajini, P. (2004). Free radical scavenging activity of an aqueous extract of potato peel. Food Chem. 85 (4): 611-16.

Sinha, A. K. (1972). Colorimetric assay of catalase. Anal. Biochem. 47: 389-394.

Slinkard, K. and Singleton, V. L. (1977). Total phenol analysis: Automation and comparison with manual methods. Am. J. Enol. Vitic. 28: 49-55.

Song, Y. and Cho, S.K. (2015). Phytol Induces Apoptosis and ROS-Mediated Protective Autophagy in Human Gastric Adenocarcinoma AGS Cells. Biochem Anal. Biochem 4: 211.

Spanova, M., and Daum, G. (2011). Squalene – biochemistry, molecular biology, process

biotechnology, and applications. Eur. J. Lipid Sci. Technol: 1-22.

Thomas, N. (2007). "Synthesis of vitamin E"In Litwack, Gerald. Vitamin E. Vitam. Horm. 76: 155-202.

Thompson W. R. (1947). Use of moving averages and interpolation to estimate median-effective dose. I. Fundamental formulas, estimation of error and relation to other methods. Vitam. Horm. 11: 115-145

Tjan, T. S. L. (2001). Squalene the miraculous essential omega 2 oil. Secrets from the sea, published on-line Natural Sources for Health. Sci. Life.

Trease, G. and Evans, W. C. (1989). A text book of pharmacognosy. Bacilluere Tinal Ltd, London.

Verma, A. R., Vijayakumar M., Chandra, M. S. and Chandana, R. V. (2009). In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. Food Chem. Toxicol. 47 (9): 2196-2201.

Wall, M. E., McClenne, M. L., Eddy, C. R., and Klump M. E. (1952). Detection of estimation of steroid sapogenins in plant tissue. Anal. Chem. 24: 137.

Wall, M. E., Krewson, C. E., Krider, M. M., Wilaman, J. J., Edyy, C. F., Cordel, G. S. and Gentry, H. S. (1954). Steroidal sapogenins VII'. Agric. Res. Serv. Circ. Aic 363: 17.

Yasmin, S., Stuti, M, Das J., Rani, M. and D'Souza D. (2011). Sub Chronic Toxicity of Arsenic Trioxide on Swiss Albino Mice. Int. J. Environ. Sci. 1.7

Zhang, Z., Gao, L., Cheng, Y., Jiang, J., Chen, Y., Jiang, H., Yu, H., Shan, A. and Cheng, B. 2014. Resveratrol, a Natural Antioxidant, Has a Protective Effect on Liver Injury Induced by Inorganic Arsenic Exposure. Biomed. Res. Int. 2014: 1-7.

Zhao, X., Cong, X., Zheng, L., Xu, L., Yin, L. and Peng, J. 2012. Dioscin, a natural steroid saponin, shows remarkable protective effect against acetaminophen-induced liver damage in vitro and in vivo. Toxicol. Lett. 214 (1): 69-80.

Zhilen, J., Mengeheng, T. and Jianming, W. (1999). The determination of flavonoids contents

in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64: 555–559.