Trop J Nat Prod Res, December 2018; 2(12):506-511 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Investigation of Chemical, Genotoxic and Haematological Properties of Secondary Metabolites from *n*-Hexane Extract of *Olax subscorpioidea* Oliv. (Olacaceae) Leaves

Akolade R. Oladipupo[1], Chinwe S. Alaribe[1]*, Olalekan T. Balogun[1], Halima T. Akere[1], Fatima A. Ayanda[1], Emuejevoke T. Toye[2], Herbert A. B. Coker[1]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, Nigeria.*
[2]*Biologix Support Services, Anthony Village, Maryland, Ikeja, Lagos, Nigeria.*

ARTICLE INFO

Article history:
Received 12 November 2018
Revised 13 December 2018
Accepted 14 December 2018
Published online 03 January 2019

ABSTRACT

Due to their phytochemical diversity, plants may exhibit various biological activities. They may be medicinal as well as toxic. As a result, investigating their toxicities is as important as investigating their medicinal properties. This study investigated the chemical, genotoxic and haematological properties of the *n*-hexane extract of *Olax subscorpioidea* leaves. The chemical investigation of the extract was done by gas chromatography-mass spectrometry (GC-MS) analysis. Genotoxic and haematological investigation were done using *in vivo* models in mice. Genotoxic effects were estimated by evaluating DNA fragmentation with agarose gel electrophoresis and diphenylamine (DPA) assays. Haematological indices were measured on an Auto Haematology Analyzer. With GC-MS analysis, eight compounds were identified in the extract. The major compounds were 9,12-octadecadienoic acid (Z, Z)- (18.00%), *n*-hexadecanoic acid (17.60%), and squalene (4.12%), which are of reported medicinal properties. Genotoxicity results indicated that the extract markedly and significantly reduced DNA fragmentation in testicular and hepatic cells in mice. Haematological analysis showed that the extract significantly increased white blood cells (WBC), lymphocytes (LYMP), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), and significantly reduced packed cell volume (PCV), haemoglobin concentration (HB), and red blood cell (RBC) count. These results indicate that the *n*-hexane extract of *Olax subscorpioidea* leaves contain compounds that possess useful pharmacologic properties, and may reduce DNA damages as well as alter haematological indices.

Keywords: Toxicity, GC-MS, phytochemicals, genotoxicity, DNA, haematological indices.

Introduction

Plants have been used for ethnomedicinal purposes in the treatment of various illnesses since antiquity and they are widely used as alternative to orthodox medicines in modern times. In addition, documentation abounds providing scientific evidences attesting to the fact that extracts from different plants possess useful bioactivities and may be used in the management of different disease conditions.[1,2] This may explain in part the reason herbal medicines are increasingly used and are recording huge sales around the world.

As the use of herbal medicines continues to increase globally, concerns surrounding their safety are also increasingly recognized.[2,3] In concomitant to their medicinal properties, herbal medicines—like the orthodox drugs—can exhibit toxicity to men.

Toxicity is defined as the degree to which a chemical substance or a particular mixture of substances can damage an organism.[4] Toxicity can occur on many different time-scales and can be classified as acute, sub-chronic and chronic. The toxic damages can be on a whole organism or its organs, tissues or cells and as a result toxicity can also be classified as; on whole organism (lethality), on organs (e.g. liver: hepatotoxicity, kidney: nephrotoxicity), on tissues (e.g. blood: haematotoxicity), on cells (cytotoxicity). Toxicity tests are done to investigate, inter alia, the lethal, reproductive, developmental, immunological and hematological effects of a substance for human use.[5]

Cytotoxicity, the toxicity of a substance to cells, is important in toxicity investigation. Cell is the fundamental unit of life; the toxic effects at cellular level are the roots of those observe at tissue, organ or systemic level. Cytotoxicity tests are usually done to evaluate a substance for unwanted cytotoxic property or anticancer potential in targeting rapidly dividing cancer cells. Two of the hallmarks of cytotoxicity are necrosis and apoptosis and these can be triggered by damage to deoxyribonucleic acid (DNA) or other genetic materials.[6] DNA contains the genetic instructions for the development and functions of a cell. Damage to DNA implies loss of the integrity of a cell which can lead to loss of cell functions or cell death and can as well initiate neoplasm and impairment of affected tissues or organs. The toxicity of a substance on DNA or other genetic material is described as genotoxicity. To estimate the genotoxic effects of a substance, the damage on DNA in cells exposed to such substance is assayed.[7]

The toxic effects of a substance on blood and its components can be described as haematotoxicity. Blood performs many important functions within the body which includes; delivery of nutrients and oxygen to cells and tissues, removal of waste products from cells and tissues, immunological functions, messenger functions and regulation of core body temperature.[8] These functions are dependent on appropriate balance of the components of the blood. A substance may interfere with blood leading to depletion or accretion of any of its

*Corresponding author. E mail: salaribe@unilag.edu.ng; aalaribe56@gmail.com
Tel: +2348037263962

Citation: Oladipupo AR, Alaribe CS, Balogun OT, Akere HT, Ayanda FA, Toye ET, Coker HAB. Investigation of Chemical, Genotoxic and Haematological Properties of Secondary Metabolites from *n*-Hexane Extract of *Olax subscorpioidea* Oliv. (Olacaceae) Leaves. Trop J Nat Prod Res. 2018; 2(12):506-511. doi.org/10.26538/tjnpr/v2i12.3

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

components which may alter the blood's biochemical properties and functions and have adverse effects. Therefore, investigation of the haematotoxicity or haematological changes induced by a substance is important.

The medicinal or toxic properties of a plant are determined by its phytochemical constituents. Most phytochemicals are secondary metabolites and the most important bioactive ones include alkaloids, flavonoids, tannins, terpenoids, and glycosides among others. These groups of phytochemicals possess diverse bioactivities and offer insight into the biological effects of a plant. To identify specific phytochemical responsible for certain bioactivity it is important to probe the individual compounds.

Olax subscorpioidea Oliv. belongs to the family *Olacaceae*. It is a tree or sometimes a many-stemmed shrub that grows up to 10 m high. *O. subscorpioidea* is widely found across Nigeria, Senegal, Zaire and Western Cameroun. It is called *Ifon* by the Yorubas, *Atu-ogili* by the Igbos and *Gwaanon kurmii* by the Hausas in Nigeria.[9] Ethnobotanical surveys have revealed that different parts of *O. subscorpioidea* is used for the management of diabetes mellitus,[10] cancer,[11] infectious diseases, mental illnesses,[12] arthritis, rheumatism, and asthma.[13] Also, researches have shown that the plant displayed antimicrobial,[14] anti-ulcer,[15] antinociceptive,[16] anti-protease,[17] and anti-depressant[16] activities. The leaves[18] and stem[14] of the plant have been reported to contain classes of bioactive compounds; among them are tannins, alkaloids, saponins, flavonoids, and glycosides. *O. subscorpioidea* has a characteristic scent which is an indication that it contains volatile aroma compounds that can be extracted as essential oils by hydrodistillation or as concrete by solvent extraction with non-polar solvents. To the best of our search, there is no documentation on the volatile extract of *O. subscorpioidea*. Therefore, the present study is aimed at extracting and profiling the volatile and non-polar secondary metabolites of *O. subscorpioidea* leaves as well as investigating their genotoxic and haematological properties.

Materials and Methods

Chemicals and Reagents

n-Hexane (BDH), normal saline, phosphate buffer saline, tween 80, trichloroacetic acid, glacial acetic acid, sulphuric acid, diphenylamine, and all other reagents used were of analytical grade.

Collection and Authentication of Plant Materials

Fresh leaves of *O. subscorpioidea* were collected from Sango-ota, Ogun State in April, 2017. The plant materials were authenticated by Mr Oyebanji at the Herbarium of the Department of Botany, University of Lagos, Akoka, where the voucher specimen (No. LUH: 7562) was deposited.

Extraction of Plant Materials

The collected leaves of *O. subscorpioidea* were air dried under shade and the dried materials ground into fine powder. The pulverized plant material (300 g) was extracted — three times — by maceration with 1.5 L of *n*-hexane at room temperature for 72 h. The extracts were combined and concentrated *in vacuo* using rotary evaporator to obtain a semi-solid sticky mass (approximately 2.0%w/w yield) herein referred to as *O. subscorpioidea* leaves *n*-hexane extract (OSLH).

Analysis of Chemical Composition

Analysis of the chemical composition of the *n*-hexane extract of *O. subscorpioidea* leaves (OSLH) was done on GC-MS (Agilent Technologies) equipped with HP-5MS capillary column (30.0 m x 0.25 mm x 0.25 µm; composed of 100% dimethyl poly-siloxane) and helium as the carrier gas at a flow rate of 2.0 mL/min. GC oven temperature was programmed as 45ºC (5 min) to 325ºC (10 min), at an increasing rate of 15ºC/min. Total GC running time was 35 min. The temperature of the injector and the interface were set at 280ºC and 350ºC, respectively. Mass spectra were recorded on an electron ionization system with ionization energy of 70 eV, and an ion source temperature of 230ºC. Identification of compounds was done by comparison of their spectra data with reference data in National Institute of Standards and Technology (NIST) library.

Toxicity Studies

Experimental Animals

Male albino mice, average weight of 21 g, were obtained from animal facility centre, College of Medicine of the University of Lagos and used for this study. The animals were kept in a well-ventilated animal house, fed with standard mice diet and water *ad libitum* and acclimatized for 14 days prior to the experiment.

Experimental Protocols

Toxicity study was carried out according to OECD[19] limit test (with some modifications) and was approved by the Health Research Ethics Committee of College of Medicine, University of Lagos with reference number; CMUL/HREC/03/18/341. The acclimatized animals were randomly split into three groups — 1, 2 and 3— of three animals each. Groups 1 and 2, the control groups, were orally administered normal saline: 0.90% w/v of NaCl in water (negative control) and 3% tween 80-normal saline (vehicle control) respectively. Group 3, the test group, was orally administered 50 mg/kg dose of OSLH (in 3% tween 80-normal saline). The animals were observed for 48 h for behavioural variations. At the end of this period, blood samples were collected by retro-orbital puncture with capillary tube into EDTA bottles, the animals were sacrificed by cervical dislocation and their livers and testes were harvested for genotoxicity studies.

Genotoxicity Studies

Qualitative Assessment of DNA Fragmentation by Gel Electrophoresis

Qualitative assessment of DNA fragmentation was carried out on agarose gel electrophoresis. Isolation of genomic DNA from the liver and testis samples was done according to Feki *et al*.[20] with some modifications. A portion (20 mg) of each organ was washed with 100 µL of phosphate buffer saline (PBS), followed by addition of 200 µL lysis buffer and 10 µL proteinase K., the resulting mixture was mixed in vortex mixer and heated in water bath at 55ºC for 2 – 3 h. To this mixture, equal volumes (100 µL) of tris-EDTA (TE) buffer and 2% sodium dodecyl sulphate (SDS) were added, vortex mixed, and centrifuged at 12,000 g for 1 min to extract the DNA. Equal volume of extracted DNA (20 µL) was loaded on 2% agarose gel, stained with ethidium-bromide DNA dye and electrophoresed at 40 V for 2 h in X0.5 tris/acetate/EDTA (TAE) buffer. DNA fragments were visualised and photographed by exposing the gel to ultraviolet (UV) trans-illumination.

Estimation of Fragmented DNA by Diphenylamine (DPA Assay)

Estimation of DNA fragmentation was carried out according to the colorimetric DPA assay described by Gibb *et al*.[21] A portion (30 mg) of each harvested organ was lysed in 0.5 mL lysis buffer and centrifuged at 10000 r.p.m. (Eppendorf) for 20 min at 4°C. The pellets were resuspended in 0.5 mL of lysis buffer. To the pellets (P) and supernatant (S), 1.5 mL of 10% trichloroacetic acid (TCA) was added and incubated at 4°C for 10 min. Subsequently, to each sample, 2 mL of DPA solution was added and incubated at room temperature for 24 h. The absorbance of the samples was then taken at 600 nm and the proportion of fragmented DNA was calculated from the following expression:

$$\% \text{ DNA fragmentation} = \frac{\text{A of frag. DNA (S)} \quad X \ 100}{\text{A of frag. DNA (S)} + \text{A of intact DNA (P)}}$$

where A is the absorbance of the sample

Assay of Haematological Parameters

The following haematological parameters, haemoglobin concentration (HB), red blood cell (RBC) count, white blood cell (WBC), packed cell volume (PCV), platelets (PLT), neutrophils (NEU), monocytes (MID), lymphocytes (LYMP), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were measured on an Auto Haematology Analyzer (MINDRAY BC-3200).

Statistical Analysis

All values are reported as the mean ± S.E.M. (standard error of mean). The values of the variables were analysed for statistically significant differences using the Student's t-test on Microsoft Excel 2010® Data Analysis tools. Differences were considered significant at $P < 0.05$.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Results and Discussion

There is increasing concern about the harmful effects of synthetic additives used in food and pharmaceutical industries and this has contributed to increasing interest in ingredients from natural sources as alternative. The use of plants volatile and aromatic extracts as ingredients in foods, drinks, and cosmetics is gaining recognition. We herein present our findings from the investigation of the chemical, genotoxic and haematological properties of the volatile and non-polar secondary metabolites of *O. subscorpioidea* leaves.

GC-MS Analysis of Chemical Composition

The results of GC-MS analysis of the chemical composition of the *n*-hexane extract of *O. subscorpioidea* leaves (OSLH) are as presented in Table 1 and Figures 1 - 2, which show the identified secondary metabolites with their retention time, molecular formula, molecular weight, percentage composition, and structures. The most abundant compounds are 9,12-octadecadienoic acid (Z,Z)- (18.0%) and *n*-hexadecanoic acid (17.6%).

9,12-Octadecadienoic acid (Z,Z)- (linoleic acid), a compound made up of 18-carbon chain with 2-double bonds in cis configuration (cis-9,12), is a polyunsaturated essential fatty acid. Fatty acids have been reported to possess antimalarial potential to inhibit the fatty acid biosynthetic machinery of *Plasmodium falciparum* parasite.[22] 9,12-Octadecadienoic acid (Z,Z)- has been shown to inhibit *Plasmodium berghei* growth by 64% using the 4-day suppressive test.[22] Linoleic acids with two conjugated double bonds (CLA: Conjugated linoleic acids) have been shown to suppress human tumor,[23] to reduce metastasis of cancers to lung tissue[24] and to possess antiatherogenic, antidiabetogenic and immune modulating properties.[25]

The leaves extract also contains *n*-hexadecanoic acid (17.6%), a fatty acid which is widely used in food and cosmetic industries. *n*-Hexadecanoic acid has shown anti-inflammatory property, Aparna et al.,[26] reported that it is a potent inhibitor of phospholipase A2, a pro-inflammatory enzyme. *n*-Hexadecanoic acid has also been reported to strongly boost metastasis in mouse models of human oral cancer.[27] Based on indication that it may increase low-density lipoproteins (LDL) levels in the blood, consumption of *n*-hexadecanoic acid has been reported to increases the risk of developing cardiovascular diseases.[28]

Squalene, a triterpene and a biochemical precursor in the biosynthesis of steroids, was identified in the leaves. Due to its biocompatibility, squalene is used as an excipient in pharmaceutical formulations. Supplementation of squalene to mice has been reported to markedly increase cellular and non-specific immune functions in a dose-dependent manner and squalene is used in conjunction with surfactants in vaccines to stimulate immune response.[29]

Octadecanoic acid, which was also identified, is widely used as surfactant and softening agent due to its bipolar nature and found wide application in cosmetic, soap, and detergent industries. Other compounds identified are long chain alkanes: nonacosane, and hentriacontane; an unsaturated aldehyde: 9,17-octadecadienal, (Z)-; and an ester: 7,10,13-hexadecatrienoic acid, methyl ester, which have unknown pharmacological property save hentriacontane which has been reported to possess anti-inflammatory property.[30]

Genotoxicity Assays

The results of genotoxicity assays of *n*-hexane extract of *O. subscorpioidea* leaves (OSLH) are as presented in Figure 3 and Table 2, which show agarose gel electrophoresis and percent DNA fragmentations of livers and testes in OSLH treated and control groups. DNA fragmentation was detected in all the groups. Agarose gel electrophoresis (Figure 3) showed the formation of smear, indicating DNA laddering, which was greater in the testes than the livers in the OSLH treated and control groups. DNA laddering represents random DNA fragmentation, a characteristic feature of apoptosis or necrosis,[31] which are binary events with consequential physiologic effects. Furthermore, the DNA fragmentation was determined quantitatively with diphenylamine (DPA) assay. The DPA assay revealed that the DNA fragmentations in hepatic and testicular cells were lower in the OSLH treated group (27.94 ± 0.41 and 49.74 ± 1.78, respectively) compared to the vehicle control group (45.80 ± 0.93 and 60.52 ± 8.49, respectively) (Table 2). These suggest that the *n*-hexane extract of *O. subscorpioidea* leaves effected marked reduction in DNA fragmentation in hepatic and testicular cells compared to the vehicle control group and indicate that the extract could reduce DNA damage induced by physiologic stress. This may be associated to the presence of bioactive secondary metabolites in the extract, particularly, *n*-hexadecanoic acid which is abundant in the extract and has been documented to possess anticancer property.[27]

Assays of Haematological Parameters

The results of the hematological study of *n*-hexane extract of *O. subscorpioidea* leaves in mice are as presented in Table 3. The results showed that the extract significantly reduced haemoglobin concentration (HB), packed cell volume (PCV), red blood cell (RBC) count, neutrophils (NEU), and monocytes (MID), and significantly increased white blood cell (WBC), lymphocytes (LYMP), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) compare to the vehicle control group. The reduction in HB and PCV mirrored the reduction in RBC which is implicative of anaemic effect.

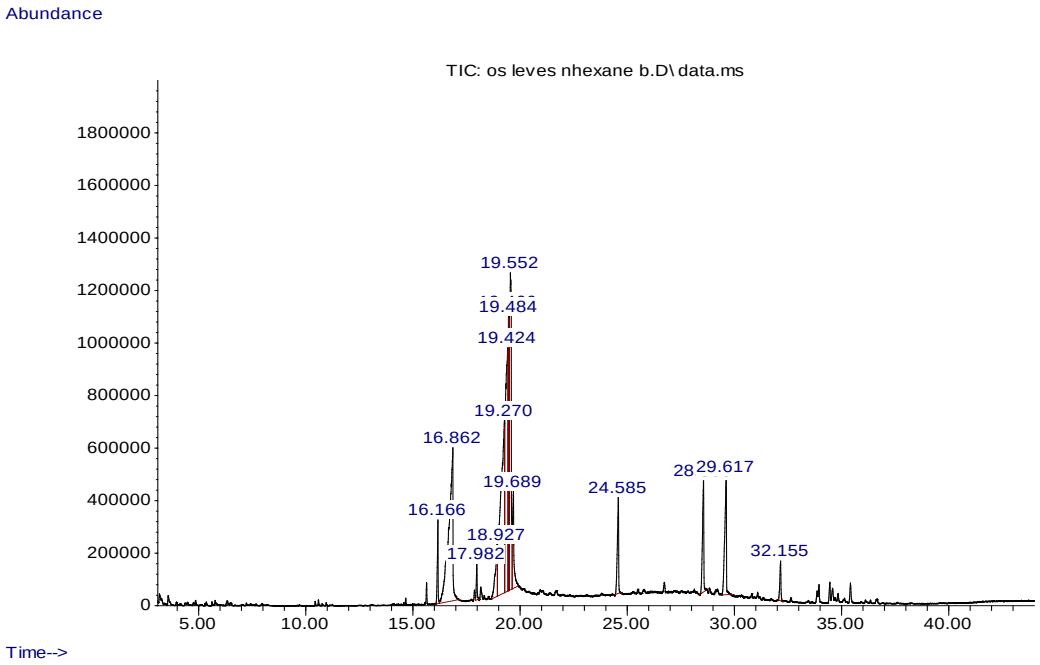

Figure 1: Total ion chromatogram (TIC) of *n*-hexane extract of *O. subscorpioidea* leaves (OSLH).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Chemical composition of *n*-hexane extract of *O. subscorpioidea* leaves (OSLH).

S/N	Ret. time (min)	Name	Molecular formula	Molecular weight(g/mol)	Area (%)
I	16.862	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.424	17.6
II	17.982	7,10,13-Hexadecatrienoic acid, methyl ester	$C_{17}H_{28}O_2$	264.403	0.84
III	18.927	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264.446	2.87
IV	19.270	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.446	18.0
V	19.689	Octadecanoic acid	$C_{18}H_{36}O_2$	284.477	4.05
VI	28.560	Squalene	$C_{30}H_{50}$	410.718	4.12
VII	29.617	Nonacosane	$C_{29}H_{60}$	408.787	5.54
VIII	32.155	Hentriacontane	$C_{31}H_{64}$	436.839	1.22

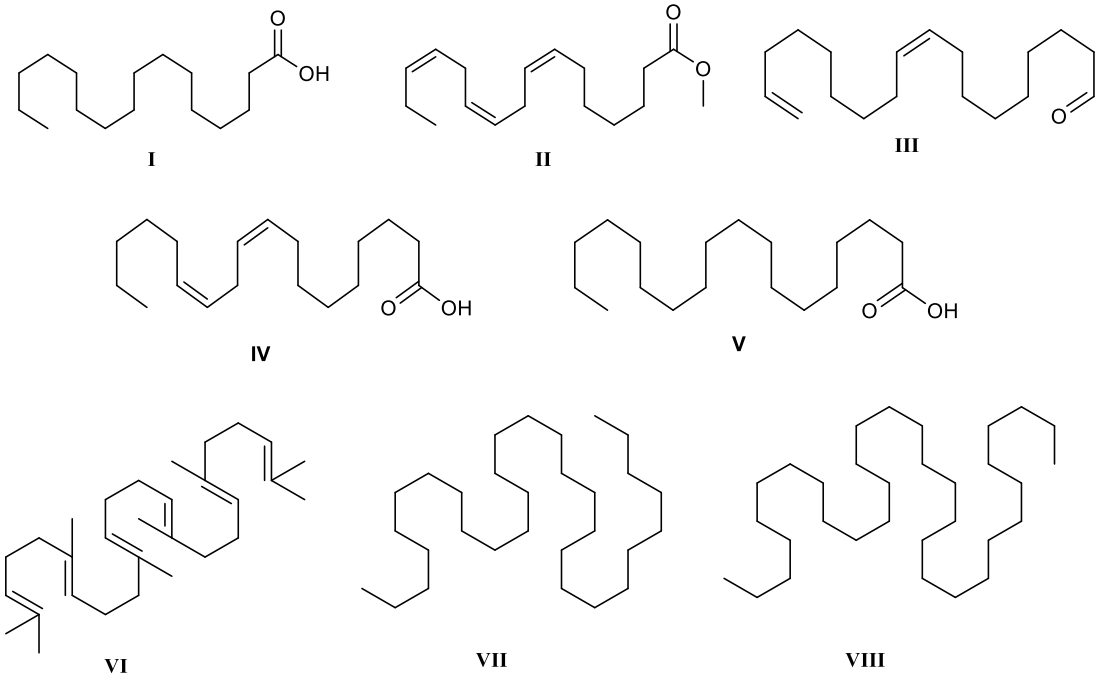

Figure 2: Structures of secondary metabolites identified in *n*-hexane extract of *O. subscorpioidea* leaves: *n*-Hexadecanoic acid (**I**), 7,10,13-Hexadecatrienoic acid, methyl ester (**II**), 9,17-Octadecadienal, (Z)- (**III**), 9,12-Octadecadienoic acid (Z,Z)- (**IV**), Octadecanoic acid (**V**), Squalene (**VI**), Nonacosane (**VII**), and Hentriacontane (**VIII**).

Elevated levels of WBC, PLT, and LYMP typically reflect the normal response of the immune system to infectious or inflammatory process.[32] These cells are part of the immune system. They identify and eliminate pathogens or other invasive substances by contact or by phagocytosis.[33] The elevated levels of these cells may be attributed to the presence of a constituent that triggers immune response in the *n*-hexane extract of *O. subscorpioidea* leaves. This is suspected to be squalene, which is reported to be used in conjunction with surfactants in vaccines to stimulate immune response.[29] Although haematological index is not definitive, these findings suggest that the *n*-hexane extract of *O. subscorpioidea* leaves should be used with caution.

Conclusion

The secondary metabolites identified in the *n*-hexane extract of *O. subscorpioidea* leaves include 9,12-octadecadienoic acid (Z, Z)-; *n*-hexadecanoic acid; squalene, and hentriacontane, which are of documented pharmacological importance. Genotoxicity assays indicated that these metabolites demonstrated reduction in DNA fragmentation in both livers and testes of treated mice, which suggests that they may reduce DNA fragmentation or augment normal DNA repair system in these organs. Haematological assays revealed that the extract altered some haematological parameters in treated mice. These findings may give credence to some of the ethnomedicinal uses of *O. subscorpioidea* leaves and recommend caution in the medicinal use of this plant.

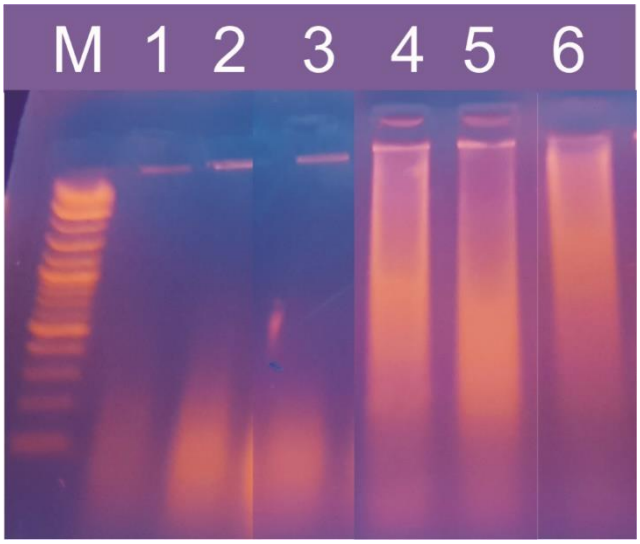

Figure 3: Agarose gel electrophoresis showing DNA fragmentation in mouse hepatic and testicular cells.
Lane M; DNA molecular weight marker, Lane 1, 2, 3; liver of negative, vehicle control and OSLH treated mice respectively; Lane 4, 5, 6; testis of negative, vehicle control and OSLH treated mice respectively.

Table 2: DNA fragmentations of livers and testes of OSLH treated and control mice.

Groups	DNA fragmentation (%)	
	Liver	**Testis**
Negative Control	56.07 ± 0.57	66.15 ± 2.22
Vehicle Control	49.74 ± 1.78	60.52 ± 8.49
OSLH	27.94 ± 0.41*	45.80 ± 0.93

Values are mean ± S.E.M. (n=3). Values with asterisk (*) are significantly different ($p < 0.05$)

Conflict of interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 3: Haematological indices of OSLH treated and control mice.

Groups	WBC (10^6/L)	HB (g/dL)	PCV (%)	RBC (10^{12}/L)	PLT (10^{12}/ cmm)	NEU (%)	LYMP (%)	MID (%)	MCV (fL)	MCH (pg/cell)	MCHC (g/dL)
Negative control	2.20 ± 0.03	14.60 ± 0.17	53.00 ± 0.61	9.30 ± 0.11	949.00 ± 10.90	44.00 ± 0.51	10.00 ± 0.12	46.00 ± 0.53	57.00 ± 0.66	15.60 ± 0.18	27.50 ± 0.32
Vehicle control	2.30 ± 0.03	14.30 ± 0.17	52.10 ± 0.60	8.10 ± 0.09	938.00 ± 10.80	45.00 ± 0.51	9.00 ± 0.10	46.00 ± 0.53	64.20 ± 0.74	17.70 ± 0.20	27.40 ± 0.32
OSLH	3.40 ± 0.04*	13.60 ± 0.16*	43.00 ± 0.61*	6.20 ± 0.10*	969.00 ± 11.20	33.00 ± 0.38*	34.00 ± 0.39*	33.00 ± 0.38*	69.00 ± 0.80*	21.90 ± 0.25*	31.60 ± 0.36*

Values are mean ± S.E.M. (n=3). Values with asterisk (*) are significantly different ($p < 0.05$).

Acknowledgements

The authors are grateful to the Central Research Laboratory, University of Lagos, for the GC-MS analysis.

References

1. Coker HAB. What has the Chemist got to do with Healthcare Delivery? Inaugural Lecture Series. Lagos: University of Lagos Press; 2005. 121 p.
2. Sasidharan S, Badakhshan MP, Rameshwar NJ, Ramanathan S. Comparative study: antimicrobial activity of methanol extracts of *Lantana camara* various parts. Pharmacogn Res. 2009; 1:348 – 351.
3. Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju G. Biological screening of medicinal plants collected from Eastern Ghats of India using *Artemia salina* (brine shrimp test). Int J Appl Sci Eng. 2006; 4:115 – 125.
4. Toxicity. (n.d) In Merriam-Webster's collegiate dictionary. [Online]. 2018 [cited 2018 Aug 2]. Available from http://www.merriam-webster.com/dictionary/toxicity
5. Davis ME and Reasor MJ. Principles of Toxicology. In: Craig CR, Stitzel RE (Eds.). Modern Pharmacology With Clinical Applications 6th Ed. Philadelphia: Lippincott Williams & Wilkins; 2003. 63 – 65 p.
6. Van Cruchten S and Van Den Brook W. Morphological and biochemical aspects of apoptosis, oncosis and necrosis. Anat Histol Embryol 2002; 31(4):214 – 223.
7. Pellevoisin C, Bouez C, Cotovio J. Cosmetic industry requirements regarding skin models for cosmetic testing. In: Marques AP, Pirraco RP, Cerqueira MT, Reis RL (Eds.). Skin Tissue Models. Amsterdam: Elsevier Inc.; 2018. 3 – 37 p.
8. Weiss C and Jelkmann W. Functions of the Blood. In: Schmidt RF, Thews G (Eds.). Human Physiology. Berlin, Heindelberg, New York: Springer; 1989. 402 – 438 p.
9. Gbadamosi IT and Erinoso SM. A review of twenty ethnobotanicals used in the management of breast cancer in Abeokuta, Ogun State, Nigeria. Afr J Pharm Pharmacol. 2016; 10(27):546 – 564.
10. Soladoye MO, Amusa NA, Raji-Esan SO, Chukwuma EC, Taiwo AA. Ethnobotanical survey of anti-cancer plants in Ogun state, Nigeria. Ann Biol Res. 2010; 1(4):261 – 273.
11. Soladoye MO, Chukwuma EC, Owa FP. An avalanche of plant species for the traditional cure of Diabetes mellitus in South-Western Nigeria. J Nat Prod Plant Resour. 2012; 2:60 – 72.
12. Ibrahim JA, Muazzam I, Jegede IA, Kunle OF, Okogun JI. Ethno-medicinal plants and methods used by Gwandara tribe of Sabo Wuse in Niger state, Nigeria, to treat mental illness. Afr J Trad Complement Altern Med. 2008; 4:211 – 218.
13. Sonibare MA and Gbile ZO. Ethnobotanical survey of anti-asthmatic plants in South Western Nigeria. Afr J Trad Complement Altern Med. 2008; 5:340 – 345.
14. Ayandele AA and Adebiyi AO. The phytochemical analysis and antimicrobial screening of extracts of *Olax subscorpioidea*. Afr J Biotechnol. 2007; 6(7):868 – 870.
15. Victoria UC, Michael UC, Johnny MU. Evaluation of the antiulcer activity of *Olax subscorpioidea* Oliv. roots in rats. Asian Pac J Trop Med. 2010; 3:13 – 16.
16. Adeoluwa OA, Aderibigbe AO, Olonode ET. Antinociceptive property of *Olax subscorpioidea* Oliv (Olacaceae) extract in mice. J Ethnopharmacol. 2014; 156:353 – 357.

17. Oyedapo OO and Famurewa AJ. Antiprotease and membrane stabilizing activities of extracts of *Fagara. Zanthoxyloids*, *Olax subscorpioides* and *Tetrapleura tetraptera* . Pharmacol Biol. 1995; 33:65 – 69.
18. Abdulazeez I, Lawal AY, Aliyu S. Phytochemical screening and antimicrobial activity of the solvents' fractionated leaves extract of *Olax subscorpioidea*. J Chem Pharm Res. 2015; 7(9):22 – 26.
19. OECD. Guidelines for the Testing of Chemicals; Test No. 425: Acute Oral Toxicity - Up-and-Down-Procedure (UDP). Paris: OECD Publishing, 2008.
20. Feki A, Saad BH, Jaballi I, Magne C, Boudawara O, Zeghal KM, Hakim A, Ali BY, Amara BI. Methyl thiophanate-induced toxicity in liver and kidney of adult rats: a biochemical, molecular and histopathological approach. Cell Mol Biol (Noisy le Grand) 2015; 63(2):20 – 28.
21. Gibb RK, Taylor DD, Wan T, O'Connor DM, Doering DL, Gerçel-Taylor C. Apoptosis as a measure of chemosensitivity to cisplatin and taxol therapy in ovarian cancer cell lines. Gynecol Oncol 1997; 65(1):13 – 22.
22. Melariri P, Campbell W, Etusim P, Smith P. *In Vitro* and *in Vivo* Antimalarial Activity of Linolenic and Linoleic Acids and their Methyl Esters. Adv Stud Biol. 2012; 4(7):333 – 349.
23. Tsuzuki T, Tokuyama Y, Igarashi M, Miyazawa T. Tumor growth suppression by α-eleostearic acid, a linolenic acid isomer with a conjugated triene system, via lipid peroxidation. Carcinogenesis 2004; 25:1417 – 1425.
24. Cesano A, Visonneau S, Scimeca JA, Kritchevsky D, Santoli D. Opposite effects of linoleic acid and conjugated linoleic acid on human prostatic cancer in SCID mice. Anticancer Res. 1998; 18:1429 – 1434.
25. Belury MA. Dietary conjugated linoleic acid in health: Physiological effects and mechanisms of action, Annu Rev Nutr 2002; 22:505 – 531.
26. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-inflammatory property of *n*-hexadecanoic acid: structural evidence and kinetic assessment. Chem Biol Drug Des. 2012; 80(3):434 – 439.
27. Pascual G, Avgustinova A, Mejetta S, Martin M, Castellanos A, Attolini CS, Burenguer A, Prats N, Toll A, Hueto JA, Bescos C, Di Croce L, Benitah SA. Targeting metastasis-initiating cells through the fatty acid receptor CD36. Nature 2017; 541:41 – 45.
28. World Health Organization. Diet, Nutrition and the Prevention of Chronic Diseases, WHO Technical Report Series 916, Report of a Joint WHO/FAO Expert Consultation. Geneva: World Health Organization, 2003. 88 p.
29. Kelly GS. Squalene and its potential clinical uses. Altern Med Rev. 1999; 4(1):29 – 36.
30. Su-Ji K, Won-Seok C, Sung-Soo K, Seong-Gyu K, Jae-Young U. Antiinflammatory Effect of *Oldenlandia diffusa* and its Constituent, Hentriacontane, through Suppression of Caspase-1 Activation in Mouse Peritoneal Macrophages. Phytother Res 2011; 25(10):1537 – 1546.
31. Ioannou YA and Chen FW. Quantitation of DNA fragmentation in apoptosis. Nucleic Acids Res 1996; 24(5): 992 – 993.
32. Abramson N and Melton B. Leukocytosis: Basics of Clinical Assessment. Am Fam Physician. 2000; 62(9):2053 – 2060.
33. Palacios I, Lozano M, Moro C. Antioxidant properties of phenolic compounds occurring in edible mushrooms. Food Chem. 2011; 128:674 – 678.