GC-MS PROFILING OF *VITELLARIA PARADOXA* LEAVES AND AMELIORATION OF HISTOPATHOLOGICAL CHANGES INDUCED IN ALBINO RATS CHALLENGED WITH *NAJA NIGRICOLIS* VENOM

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Abstract: Medicinal plants are an indispensable resource and despite the contemporary progresses recorded in pharmaceuticals and drug developments, they remain an invaluable source of natural products, a basis for novel drug discovery. This study employed Gas chromatography-mass spectroscopy (GC-MS) to characterize the bioactive compounds present in the methanolic leaf extract of *Vitellaria paradoxa*. Analysis of the bioactive compounds from *Vitellaria paradoxa* by GC-MS analysis revealed 25 peaks and a total of 23 compounds were identified. Vast majority of the compounds have been previously reported to have important pharmacological activities. The crude extract of *V. paradoxa* leaves ameliorated histopathological damages induced in the liver, brain and kidney of albino rats challenged with *N. nigricollis* venom when compared with the experimental control group. The present study identified an array of bioactive compounds present in *V. paradoxa*, established the snake venom detoxifying potential of the plant, and reported the ethno-botanical uses of *V. paradoxa* in the treatment of other medical exigencies.

Keywords: *Vitellaria paradoxa*, GC-MS analysis, Bioactive compounds, *N. nigricolis*, Snakebite envenomation, Neglected tropical diseases.

Introduction

Traditional medicine continues to play an important role in healthcare system since over 80 % of the population in the third world countries relies on traditional medicine (Gomathi et al., 2015). The practice of traditional medicine has been an important component of healthcare systems in the rural population basically as a result of limited access to modern medicine and most of these medicinal plants are found in the surroundings of the villages and might be threatened by unsustainable collection and harvest practice (Rakotoarivelo et al., 2015). Although these medicinal plants contain substances that can be used for therapeutic purposes and can also serve as precursors for the synthesis of useful drugs (Sofowora et al., 2013), empirical work is lacking to back this assertion. Therefore, the identification, isolation and characterization of bioactive compounds from the extracts of medicinal plants is of major importance and provides additional information for further pharmacological studies since the mode of action of plants producing therapeutic effects can also be better investigated (Brusotti et al., 2014).

Snakebite envenomation is a serious public health problem that affects about five million people especially in developing tropical and sub-tropical countries, with nearly 100,000 yearly deaths (Williams et al., 2018). *Naja nigricollis* (the black-necked spitting cobra) are prevalent and widely distributed in Africa. These elapids whose venom have been reported to contain neurotoxic and cytotoxic proteins and peptides (Warrell, 2010) have severe implications to health as envenomation leads to blistering, swelling, and necrosis (Abubakar et al., 2006).

V. paradoxa is a perennial plant of the Sapotaceae family belonging to the division: Magnoliophyta and specie of *paradoxa*. *V. paradoxa* grows naturally and commonly found in the wild dry savanna across West African countries including Cameroon, Congo, Ghana and Nigeria (IUCN, 2014). *V. paradoxa* C. F. Gaertn is used in traditional medicine for the treatment of various ailments, including dysentery, stomach ailments, cutaneous infection, inflammation, diarrhoea, dysentery, diabetes mellitus, microbial infection, and fever (Ayankunle et al., 2012). It is generally protected and venerated because of the economic value of the shea butter extracted from the fermented kernel (Sanou and Lamien, 2011).

Previously, reports have shown that the methanolic extract of *V. paradoxa* stem bark has significant anti-inflammatory and anti-arthritic effects in Carrageenan-induced inflammation and Complete Freund's Adjuvant (CFA)-induced arthritic animal model (Foyet et al., 2015). The methanolic extract of *V. paradoxa* leaves has been demonstrated to be bactericidal and fungicidal on *Staphylococcus aureus* and *Aspergillus niger*, *Candida albicans* respectively (Olaleye et al., 2015). Also, the anti-microbial (El-Mahmood et al., 2008), anti-mycotic (Ahmed et al., 2009) and anti-diarrhoeal (Abubakar et al., 2013) properties of *V. paradoxa* have been reported.

The identification of the phyto-constituents of *V. paradoxa* leaves was previously carried out by reagent-based phyto-chemical analysis and this plant has shown remarkable medicinal potential in treating various infectious diseases (Olaleye et al., 2015). With the significant development that is occurring in metabolomics for biology and natural product research, GC-MS analysis is gaining relevance from both targeted and untargeted analytical perspectives (Goulitquer et al., 2012). The recent rapid development of a broad range of analytical platforms that combines modern instrumental analytical

approaches has enormously improved our understanding of metabolomics and also increases the coverage of detected metabolites that cannot be achieved by single-analysis techniques such as GC or MS alone (Zhang et al., 2012).

Thus, in the current absence of any published report on the profiling of *V. paradoxa* leaves by GC-MS, this study analysed the bioactive compounds in the leaves of *V. paradoxa* using GC-MS, a more advanced and refined technique that provides key technological platforms for the profiling of secondary metabolites that could be exploited for precise drug-targeting against different infectious diseases.

Materials and Methods

Sample collection and preparation

The leaves of *V. paradoxa* plant were collected from the wild in Zaria, Kaduna State-Northern Nigeria. The plant was identified the Herbarium Unit of the Department of Botany, Ahmadu Bello University, Zaria-Nigeria with voucher number: 9540. The plant sample was shade-dried, pulverized into coarse powdery form and kept for further analysis.

Hydro-alcoholic extraction of V. paradoxa leaves

500g of the pulverized *V. paradoxa* leaves were weighed and subjected to extraction in a conical flask with 1.2 L of 70 % methanol for four days with gentle agitation in orbital shakers. The recovered extracts were filtered using Whatman filter papers and the filtrate was evaporated to dryness in a HH–S digital thermostatic water bath pre-set at 50 °C. The crystals obtained were preserved at 4 ˚C for further analysis.

GC-MS analysis

GC-MS analysis of the crude extracts of *V. paradoxa* leaves was performed using Shimadzu GC-MS - QP - 2010 plus system with RTx - 5 Sil MS column (30 m X 0.25 mm, 0.25). The operating conditions of the column were as follows: oven temperature program was from 80 °C to 250 °C at 10 °C/min withhold time of 3 min and from 250°C to 280°C at 15°C/min withhold time of 5 min, and the final temperature was kept for 18 min. The injector temperature was maintained at 260 °C with pressure of 85.3 kPa and linear velocity of 40.5 cm/sec. The ion source temperature was 230 °C with scan mass range of m/z 40-600, scan interval of 0.50 sec and interface line temperature was 270 °C. The volume of injected sample was 8.0 µL and the total flow was 16.3mL/min with column flow of 1.21 mL/min, purge flow 3.0 mL/min and split ratio: 10.0. The GC-MS analysis was carried out at the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi Campus, India.

Identification of bioactive compounds

The identification of components was carried out by comparing the spectra with the internal standards on National Institute Standard and Technology (NIST-08) and WILLEY-8 libraries. The relative abundance level of each component was calculated as percentages by comparing its average peak area to the total area. A web-based search for the biological activities of the bioactive compounds identified by GC-MS analysis was also carried out and the results obtained have been documented.

Animal source

Albino rats weighing 151-250 g were obtained from the Federal University of Lokoja, Kogi State, Nigeria. The animals were acclimatized for two (2) weeks at the animal house facility situated at the Department of Biochemistry, Kogi State University, Anyigba, Nigeria.

Snake Venom source

The lyophilized *N. nigricollis* venom was a generous gift from Mr. Ebinbin Ajagun of the National Biotechnology Development Agency (NABDA), Bayelsa State, Nigeria.

Administration of N. nigricolis venom and treatment with V. paradoxa

The venom was pre-incubated with the extract in an equal volume ratio (1:1) at a calculated dosage of 8mg/kg body weight for venom and extract respectively. Intramuscular administration of the mixture to rats in group 1 was done 30 min after the pre-incubation step. The experimental control groups (group 2 and 3) were administered the venom alone and extract alone respectively. Four (4) hours following envenomation, the liver, kidney and brain tissues were excised from each group of animals. These organs were initially preserved in 10% formalin for histopathology analysis. The liver, kidney and brain were fixed in 10% formalin until ready for use. After fixation, the brain and the liver were cut in transverse sections while the kidneys were cut in longitudinal sections. These specimens were suspended in absolute alcohol and absolute xylene for 4 days and embedded in paraffin. Sections were cut at 3.5µm, stained with Haematoxylin and Eosin (HE) and analysed by light microscopy.

Results and Discussions

GC-MS analysis of crude methanolic extract of V. paradoxa leaves

Evidence on the vast experiential use of *V. paradoxa* by the traditional healers in treating some infectious diseases have necessitated our choice of this plant for further evaluation having proven efficacious in combating a range of medical exigencies (Abubakar et al., 2013, Ahmed et al., 2009, El-Mahmood et al., 2008, Foyet et al., 2015).

The chromatogram of crude methanolic extract of *V. paradoxa* leaves showed 25 peaks of volatile compounds (Figure 1), and on comparison with standards in the National Institute Standard and Technology (NIST)/WILLEY 08 libraries, 23 out of the 25 bioactive compounds were identified. The active principles with their respective retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (peak area (%) are reported as shown in Table 1. Out of the 23 identified bioactive compounds, 13 possess documented biological activities based on previously published works (Table 2).

The most abundant bioactive compound identified in the crude methanolic extract of *V. paradoxa* leaves is 1,3,4,5-tetrahydroxy-cyclohexane-carboxylic acid with 17.37% peak area followed by 1,5-anhydro-6-deoxyhexo-2,3-diulose with 5.36% peak area and 1,2,3-benzenetriol with 4.55% peak area (Table 1).

Antioxidants are known to play a vital role in ameliorating the effects of neurotoxic substances such as snake venom enzymes. Hence, the presence of identified bioactive compounds in *V. paradoxa* leaves with documented antioxidant property (Table 1) could probably be responsible for the prevention of oxidative damage caused in snake envenomed victims.

The phenolic compound, 1, 2, 3-benzenetriol identified in the crude methanolic extract of *V. paradoxa* leaves has been reported to have anti-oxidant, anti-septic, anti-bacterial, anti-dermatitis, fungicide, pesticide and anti-mutagenic properties (Song et al., 2007, Vadivel and Gopalakrishnan, 2011). The antioxidant property of this compound explains the ability of the plant to serve as anti-venom.

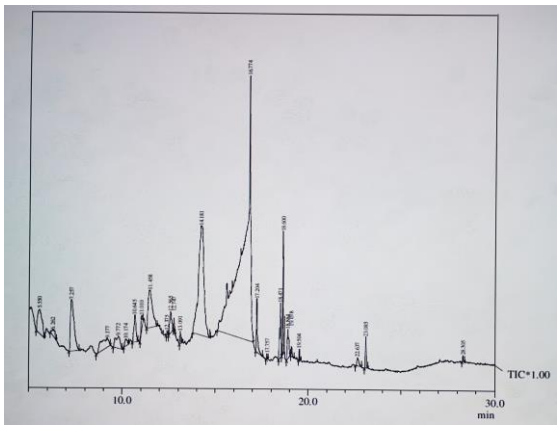

Fig. 1: The gas chromatogram of the methanolic extract of *V. paradoxa* leaves. The chromatogram shows twenty-five (25) peaks that indicates the presence of twenty-five (25) phyto-constituents. The relative amount of each component was calculated as percentages by comparing its average peak area to the total area.

Other bioactive compounds identified in the crude methanolic extract of *V. paradoxa* leaves with anti-oxidant activity includes; n-hexadecanoic acid, Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester, 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro -4,4,7a-trimethyl-, 9-Octadecenoic acid (Z),-methyl ester, and Phytol while 1,2,3-benzenetriol, 1,2-benzenedicarboxylic acid, dioctyl ester, 2(4H)-Benzofuranone, 5,6,7,7 a-tetrahydro -4,4,7 a- trimethyl -, 9-octadecenoic acid (Z)-, and phytol possesses anti-microbial activity (Duke and Bogenschutz, 1994).

Among the identified bioactive compounds, Octadecatrienoic acid and its derivatives have been previously reported to have anti-inflammatory, anti-bacterial, anti-convulsant, anti-pyretic, analgesic, anaesthetic, allergenic, anti-oxidant, anti-septic and anti-staphilococcus properties (Duke and Bogenschutz, 1994). Similarly, hexadecanoic acid (Palmitate) and its derivatives that were identified in the leaf extracts have been demonstrated to have anti-inflammatory activity through competitive inhibition of PLA_2 (Vickers et al., 2009). In a study of the anti-inflammatory property of n-hexadecanoic acid, it was reported that the competitive inhibition of PLA_2 is one of the ways to control inflammation (Aparna et al., 2012). Palmitate is also suggested to specifically enhance intestinal uptake of retinol - a very essential metabolite (Liu et al., 2013).

The derivatives of octadecenoic acid were also reported to be very strong synthetic inhibitors of neurotoxins (Grasso et al., 1993). The unsaturated fatty acids found in *V. paradoxa* extract would help in maintaining the cell integrity which in-turn prevents the distribution of venom components from the bite site (Lahousse et al., 2006).

Squalene- an intermediate metabolite in the synthesis of cholesterol (Mazein et al., 2013), was also identified from the crude methanolic extracts of *V. paradoxa* leaves. Notably, squalene plays a role in modulating molecular packing and lateral organization (i.e., domain formation) in the membranes of archaea analogous to that of cholesterol in eukaryotic membranes (Gilmore et al., 2013). This property may be responsible for the protection offered by *V. paradoxa* leaf extract in ameliorating incidence of snake bite. Squalene has also been demonstrated to have detoxification and xenobiotic properties and has tendency to attach to non-ionized substances since it is non-polar. Up to date, the anti-cancer, anti-oxidant, drug carrier, detoxifier, skin hydrating, emollient and other biological activities of squalene have been reported (Kim and Karadeniz, 2012).

The collective anti-inflammatory and antioxidant properties of the different bioactive compounds identified in the methanolic extract of *V. paradoxa* leaves scientifically explains its use in traditional medicine against vast medical exigencies such as snake bite and other infectious diseases of medical importance.

Histopathological analysis

Photomicrographs for the analysis on the histological changes observed in the different tissues excised from the respective groups of albino rats are shown in Fig. 2, Fig. 3 and Fig. 4.

Organ dysfunction has been ascribed to the effects of necrotic and cytotoxic components of snake venoms (cyto/cardio-toxins, necrotic and haemorrhagic factors/toxins and several enzymes) including a major class of enzyme called phospholipases (Conlon et al., 2013).

A comparative histological analysis of liver tissues excised from the respective groups of experimental rats (extract alone, venom alone, venom and extract) revealed convincing evidence that the liver histo-architecture was preserved, and the hepatocytes appear viable in the tissues of rats that received the extract alone (Fig. 2A). However, in the group that received the venom alone (Fig. 3A), we observed vascular and sinusoidal congestion, enlarged sinusoids, early stage of nuclear pyknosis and focal necrosis of hepatocytes. Interestingly, in the group of rats that received the extract and venom in combination (Fig. 4A), there was darkening of nuclear material of some hepatocytes, early stage of pyknosis and the hepatocytes seemed to undergo a delayed-type degeneration characterized by gradual focal necrosis and sinusoidal congestion.

In the same way, we analysed and compared the histopathological data for the brain tissues excised from the respective groups of rats and observed a preserved histo-architecture for the brain tissues (Fig. 2B and Fig 4B) except for the significant macrophage infiltration in the tissues of rats that received the venom alone (Fig. 3B). When we compared the kidney tissues excised from the respective experimental groups of rats, we observed a preserved renal histo-architecture with a mild infiltration by macrophages in the tissues of rats that received the extract alone (Fig. 2C). But in the group of rats that received the venom alone, there is evidence of

macrophage infiltration even as renal cortex histo-architecture seems to be preserved (Fig. 3C). However, in the group of rats that received the extract and the venom in combination, there was evidence of a preserved histo-architecture of the renal cortex despite the tubular necrosis and mild macrophage infiltration that was observed (Fig. 4C).

Table 1: GC-MS spectral analysis of the extract of *V. paradoxa* leaves

S/N	RT (min)	Compound name	MF	MW	Peak area (%)
1	5.550	5H- 1,4-dioxepin, 2,3-dihydro- 2,5-dimethyl-	$C_7H_{12}O_2$	128	2.96
2	6.262	Unknown	-	-	0.58
3	7.257	1,5-anhydro-6-deoxyhexo-2,3-diulose	$C_6H_8O_4$	144	5.36
4	9.177	1,2-Benzenediol	$C_6H_6O_2$	110	2.27
5	9.772	2H-Pyran-3,4,5-triol, tetrahydro-2-methoxy-6- methyl-	$C_7H_{14}O_5$	178	1.60
6	10.174	3-Decenoic acid, (E) -	$C_{10}H_{18}O_2$	170	0.50
7	10.645	2H-Pyran-3,4,5-triol, tetrahydro-2-methoxy-6- methyl-	$C_7H_{14}O_5$	178	1.59
8	11.010	Beta-D-glucopyranose-1,6-anhydro-	$C_6H_{10}O_5$	162	0.67
9	11.458	1,2,3-benzenetriol	$C_6H_6O_3$	126	4.55
10	12.375	1,2,3,6-Tetrahydropyridine-1-methyl-5-phenyl-	$C_{12}H_{15}N$	173	0.13
11	12.565	Beta-D-glucopyranose, 1,6- anhydro-	$C_6H_{10}O_5$	162	1.23
12	12.747	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	180	0.24
13	13.091	3,6-dimethyl-3-Octene-2,7-dione	$C_{10}H_{16}O_2$	168	0.29
14	14.181	1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid	$C_7H_{12}O_6$	192	17.37
15	16.774	Unknown	-	-	52.49
16	17.204	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.50
17	17.757	Methyl-17,18-dideuterioocta decanoate	$C_{19}H_{36}D_2O_2$	300	0.09
18	18.471	9-Octadecenoic acid (Z), -methyl ester	$C_{19}H_{36}O_2$	296	1.84
19	18.600	Phytol	$C_{20}H_{40}O$	296	1.17
20	18.884	9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	282	1.23
21	19.078	n-hexadecanoic acid	$C_7H_{18}OSi$	256	0.27
22	19.504	Tert-butyl (methoxy) dimethylsilane	$C_{19}H_{38}O_4$	146	0.16
23	22.637	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl) ethyl ester	$C_{15}H_{24}$	390	0.74
24	23.085	1,2-benzenedicarboxylic acid, dioctyl ester	$C_{24}H_{38}O_4$	330	0.44
25	28.305	Longifolene	$C_7H_{12}O_2$	204	0.14

KEY: RT: Retention time, MF: Molecular formula, MW: Molecular weight.

Table 2: Biological activities of the bioactive compounds identified in *V. paradoxa*

S/N	Compound	MF	Compound nature	**Activities
1	1,5-anhydro-6-deoxyhexo-2,3-diulose	$C_6H_8O_4$	Glycoside	Preservative
2	1,2-Benzenediol	$C_6H_6O_2$	Aromatic alcohol	Anti-cancer (Breast), Antioxidant, Pesticide
3	1,2,3-benzenetriol	$C_6H_6O_3$	Aromatic alcohol	Anti-oxidant, Antiseptic, Anti-bacterial, Antidermatitis, Fungicide, Pesticide, Antimutagenic, dye, Candidice. (Akhbari et al., 2012)
4	1,2,3,6-Tetrahydropyridine,1-methyl-5-phenyl-	$C_{12}H_{15}N$	Aromatic compound	A neurotoxin and produces a partial model of Parkinson's disease (PD). (Yang et al., 2008)
5	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	Sugar moiety	Analgesic, Anti-diabetic, Anti-bacterial, Anti-fungal, (Moorthy and Boominathan, 2011) Anti-algal effect, Anti-oxidant (Akhbari et al., 2012, Yang et al., 2008).
6	1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid	$C_7H_{12}O_6$	Acidic compound	Anti-hepatitis B virus, (Wang et al., 2009) Anti-obesity, (Cho et al., 2010) Radio-protective effects, (Yildiz et al., 2008) Improves cardiovascular diseases (Park, 2009), Anti-inflammatory, (Hayden and Ghosh, 2004), Anti-microbial.
7	n-hexadecanoic acid	$C_{16}H_{32}O_2$	Palmitic acid	Antioxidant, hypo-Cholesterolemic, Nematocidal, Pesticidal, Hemolytic, anti-androgenic and 5-alpha reductase inhibitor.
8	9-Octadecenoic acid (Z), -methyl ester	$C_{19}H_{36}O_2$	Fatty acid ester	Anti-oxidant,Anti-carcinogenic, endogenous receptor ligand, dermatitigenic, flavour (Asghar et al., 2011, Hema et al., 2011)
9	Phytol	$C_{20}H_{40}O$	Diterpene alcohol	Anti-microbial, Anti-cancer, Anti-inflammatory, Antioxidant, Hypo-cholesterolemic, Anti-diuretic.
10	9-Octadecenoic acid (Z)	$C_{18}H_{34}O_2$	Fatty acid	Anti-androgenic, Lipoxygenase inhibitor, Allergenic, Flavour, Hypo-cholesterolemic, Insectifuge, Irritant, Percuteneo-stimulant, Perfumery, Anti-bacterial and Propecic.
11	1,2-benzene dicarboxylic acid, dioctyl ester	$C_{24}H_{38}O_4$	Acid ester	Anti-fouling, Anti-microbial. Inhibits human platelet phospholipase A2 (Labow et al., 1988), Rapidly increases protein phosphorylation in Hela cells. (Lahousse et al., 2006)
12	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{15}H_{24}$	Fatty acid ester	Hemolytic, Pesticide, Flavour, Antioxidant.
13	Longifolene	$C_7H_{12}O_2$	Sesquiterpene	Anti-inflammatory.

** Activities source: Dr. Duke's Phyto-chemical and Ethno-botanical Database (Abubakar et al., 2013).

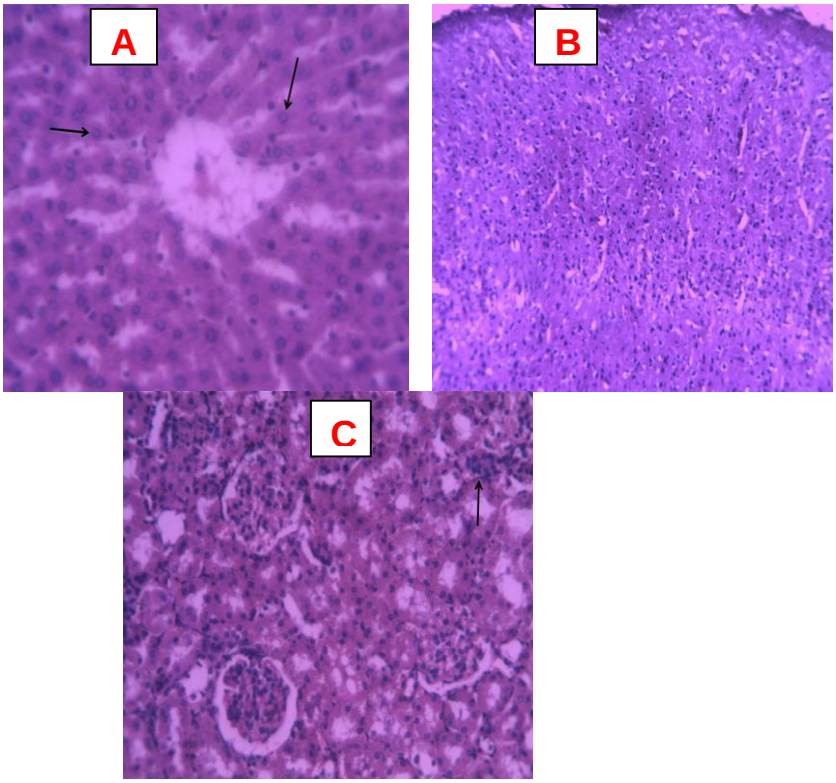

Fig. 2: Histopathological lesions observed in albino rats in group 3, administered the extract alone. (A): Section of liver tissue (×400): there is evidence of preserved liver histo-architecture, and the hepatocytes appear viable (black arrow). (B): Section of cerebral cortex (×100): cerebral histo-architecture is preserved; thus, no significant histopathological changes were observed. (C): Section of kidney (×250): renal histo-architecture is preserved; however, mild infiltration by macrophages was observed (black arrow).

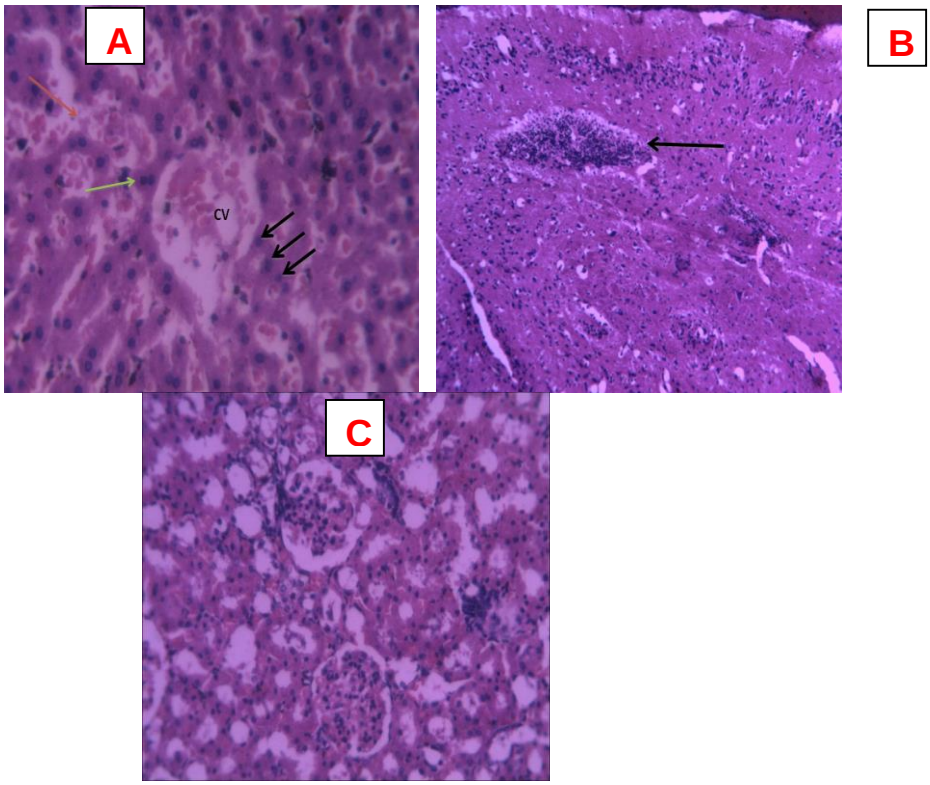

Fig. 3: Haematoxylin and Eosin staining of the different tissues excised from rats following the administration of the venom alone at 8 mg/kg body weight. (A): Section of liver tissue (×400): there is evidence of vascular and sinusoidal congestion and enlarged sinusoids (red arrow); early stage of nuclear pyknosis (green arrow) and focal necrosis of hepatocytes (black arrow).

(B): Section of cerebral cortex (×100): there is evidence of significant macrophage infiltration (black arrow). (C): Section of kidney tissue (x400): although renal cortex histo-architecture seems to be preserved, there is evidence of macrophage infiltration (mif).

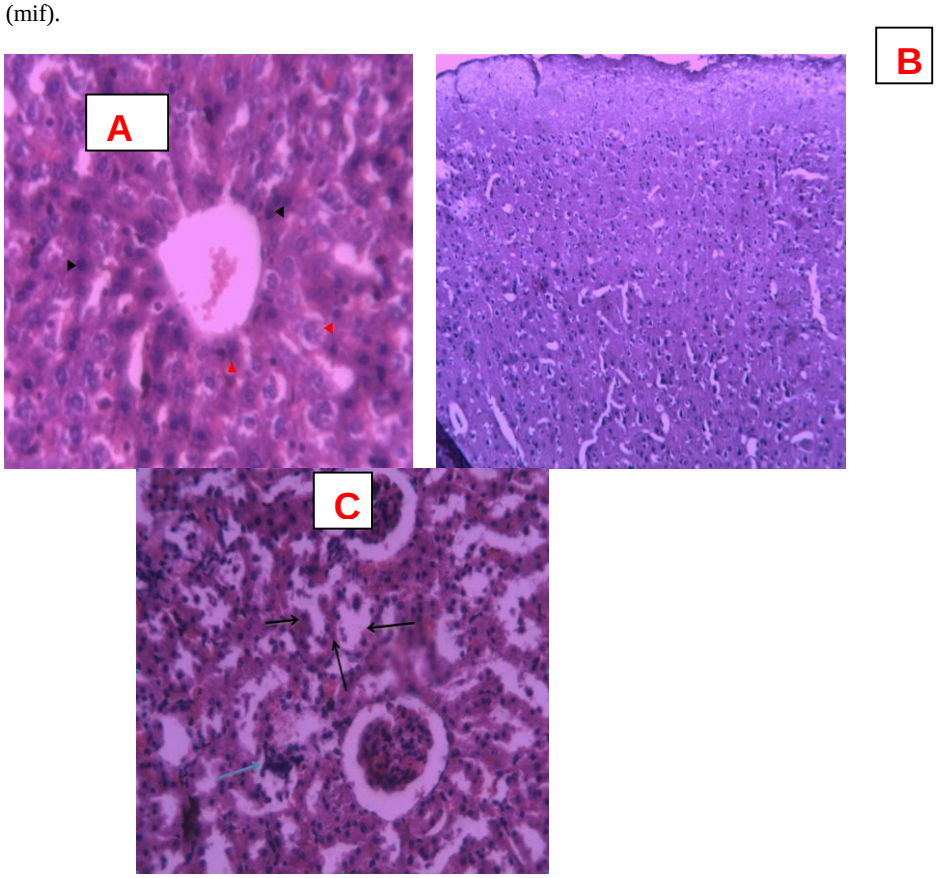

Fig. 4: Haematoxylin and Eosin staining of the different tissues excised following the administration of 1:1 volume ratio of *N. Nigricolis* venom and *V. paradoxa* leaf extracts. (A) Section of liver (×400): there is evidence of sinusoidal congestion; darkening of nuclear material of some hepatocytes, early stage of pyknosis (black arrow); focal necrosis of some hepatocytes (red arrow). (B) Section of cerebral cortex (×100): no significant histo-pathological changes were observed. (C) Section of kidney (×400): there is evidence of tubular necrosis (black arrow) and macrophage infiltration (blue arrow).

We studied the pathology of *N. nigricollis* envenomation in albino rats and histological examination of the selected organs revealed drastic degeneration of the tissues. Consistent with our observation is a report indicating that renal dysfunction is very common following viper bites (Torrez et al., 2014). These changes may be as a result of leakage of membrane caused by the action of phospholipases present in the venom. However, we have shown the efficacy of *V. paradoxa* leaf extracts in abrogating these tissue degenerations when rats were treated with the plant extract.

Conclusion

Herein, we identified an array of bioactive compounds present in the leaves of *V. paradoxa* and also reported their documented ethno-botanical uses in the treatment of a wide range of medical exigencies. We have also shown scientific evidence of *N. nigricolis* venom detoxification by *V. paradoxa.* This report could be exploited for precise drug targeting against various pathological conditions.

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Conflict of Interest

No conflict of interest.

References

Abubakar, K, Abdulkadir, R, Etuk, E & Famoriyo, P 2013. Evaluation of the Antidiarrhoeal Effect of Vitellaria Paradoxagaertn F (Sapotaceae) Stem Bark Extract. *Evaluation*, 15.

Abubakar, M, Balogun, E, Abdurahman, E, Nok, A, Shok, M, Mohammed, A & Garba, M 2006. Ethnomedical Treatment of Poisonous Snakebites: Plant Extract Neutralized Naja Nigricollis. Venom. *Pharmaceutical biology*, 44**,** 343-348.

Ahmed, R, Sani, A & Igunnugbemi, O 2009. Antifungal Profiles of Extracts of Vitellaria Paradoxa (Shea-Butter) Bark. *Ethnobotanical leaflets*, 2009**,** 2.

Akhbari, M, Batooli, H & Kashi, FJ 2012. Composition of Essential Oil and Biological Activity of Extracts of Viola Odorata L. From Central Iran. *Nat Prod Res*, 26**,** 802-9.

Aparna, V, Dileep, KV, Mandal, PK, Karthe, P, Sadasivan, C & Haridas, M 2012. Anti-Inflammatory Property of N-Hexadecanoic

Acid: Structural Evidence and Kinetic Assessment. *Chem Biol Drug Des*, 80**,** 434-9.

Asghar, SF, Habib-ur-Rehman, H-u-R, Choudahry, M & Atta-ur-Rahman, A-u-R 2011. Gas Chromatography-Mass Spectrometry (Gc-Ms) Analysis of Petroleum Ether Extract (Oil) and Bio-Assays of Crude Extract of Iris Germanica.

Ayankunle, A, Kolawole, O, Adesokan, A & Akiibinu, M 2012. Antibacterial Activity and Sub-Chronic Toxicity Studies of Vitellaria Paradoxa Stem Bark Extract.

Brusotti, G, Cesari, I, Dentamaro, A, Caccialanza, G & Massolini, G 2014. Isolation and Characterization of Bioactive Compounds from Plant Resources: The Role of Analysis in the Ethnopharmacological Approach. *J Pharm Biomed Anal*, 87**,** 218-28.

Cho, AS, Jeon, SM, Kim, MJ, Yeo, J, Seo, KI, Choi, MS & Lee, MK 2010. Chlorogenic Acid Exhibits Anti-Obesity Property and Improves Lipid Metabolism in High-Fat Diet-Induced-Obese Mice. *Food Chem Toxicol*, 48**,** 937-43.

Conlon, JM, Attoub, S, Arafat, H, Mechkarska, M, Casewell, NR, Harrison, RA & Calvete, JJ 2013. Cytotoxic Activities of [Ser(4)(9)]Phospholipase a(2) from the Venom of the Saw-Scaled Vipers Echis Ocellatus, Echis Pyramidum Leakeyi, Echis Carinatus Sochureki, and Echis Coloratus. *Toxicon*, 71**,** 96-104.

Duke, J & Bogenschutz, MJ 1994. *Dr. Duke's Phytochemical and Ethnobotanical Databases*, USDA, Agricultural Research Service Washington, DC.

El-Mahmood, A, Doughari, J & Ladan, N 2008. Antimicrobial Screening of Stem Bark Extracts of Vitellaria Paradoxa against Some Enteric Pathogenic Microorganisms. *African Journal of Pharmacy and Pharmacology*, 2**,** 089-094.

Foyet, HS, Tsala, DE, Bodo, JZE, Carine, AN, Heroyne, LT & Oben, EK 2015. Anti-Inflammatory and Anti-Arthritic Activity of a Methanol Extract from Vitellaria Paradoxa Stem Bark. *Pharmacognosy research*, 7**,** 367.

Gilmore, SF, Yao, AI, Tietel, Z, Kind, T, Facciotti, MT & Parikh, AN 2013. Role of Squalene in the Organization of Monolayers Derived from Lipid Extracts of Halobacterium Salinarum. *Langmuir*, 29**,** 7922-30.

Gomathi, D, Kalaiselvi, M, Ravikumar, G, Devaki, K & Uma, C 2015. Gc-Ms Analysis of Bioactive Compounds from the Whole Plant Ethanolic Extract of Evolvulus Alsinoides (L.) L. *J Food Sci Technol*, 52**,** 1212-7.

Goulitquer, S, Potin, P & Tonon, T 2012. Mass Spectrometry-Based Metabolomics to Elucidate Functions in Marine Organisms and Ecosystems. *Mar Drugs*, 10**,** 849-880.

Grasso, P, Heindel, JJ, Powell, CJ & Reichert Jr, LE 1993. Effects of Mono (2-Ethylhexyl) Phthalate, a Testicular Toxicant, on Follicle-Stimulating Hormone Binding to Membranes from Cultured Rat Sertoli Cells. *Biology of reproduction*, 48**,** 454-459.

Hayden, MS & Ghosh, S 2004. Signaling to Nf-Kappab. *Genes Dev*, 18**,** 2195-224.

Hema, R, Kumaravel, S & Alagusundaram, K 2011. Gc/Ms Determination of Bioactive Components of Murraya Koenigii. *Journal of American Science*, 7**,** 80-83.

IUCN 2014. The Iucn Red List of Threatened Species. Version 2014.3. *IUCN*, 12**,** 20.

Kim, SK & Karadeniz, F 2012. Biological Importance and Applications of Squalene and Squalane. *Adv Food Nutr Res*, 65**,** 223-33.

Labow, RS, Meek, E, Adams, GA & Rock, G 1988. Inhibition of Human Platelet Phospholipase A2 by Mono (2-Ethylhexyl) Phthalate. *Environmental Health Perspectives*, 78**,** 179-183.

Lahousse, SA, Beall, SA & Johnson, KJ 2006. Mono-(2-Ethylhexyl) Phthalate Rapidly Increases Celsr2 Protein Phosphorylation in Hela Cells Via Protein Kinase C and Casein Kinase 1. *Toxicological Sciences*, 91**,** 255-264.

Liu, Y, Shaw, J-J, Swaisgood, HE & Allen, JC 2013. Bioavailability of Oil-Based and B-Lactoglobulin-Complexed Vitamin a in a Rat Model. *International Scholarly Research Notices*, 2013.

Mazein, A, Watterson, S, Hsieh, WY, Griffiths, WJ & Ghazal, P 2013. A Comprehensive Machine-Readable View of the Mammalian Cholesterol Biosynthesis Pathway. *Biochem Pharmacol*, 86**,** 56-66.

Moorthy, V & Boominathan, M 2011. The Antimicrobial Activities of Crude Extracts and Fraction of Psidium Guajava and Azadirachta Indica against Staphylococcus Aureus in Chronic Disease Affected Patients. *International Journal of universal pharmacy and life sciences*, 1**,** 2249-6793.

Olaleye, O, Adetunji, C & Kolawole, O 2015. Identification of Phytochemical Constituents of the Methanolic Extract of Vitellaria Paradoxa Responsible for Antimicrobial Activity against Selected Pathogenic Organisms. *SMU Medical Journal***,** 177-190.

Park, JB 2009. 5-Caffeoylquinic Acid and Caffeic Acid Orally Administered Suppress P-Selectin Expression on Mouse Platelets. *J Nutr Biochem*, 20**,** 800-5.

Rakotoarivelo, NH, Rakotoarivony, F, Ramarosandratana, AV, Jeannoda, VH, Kuhlman, AR, Randrianasolo, A & Bussmann, RW 2015. Medicinal Plants Used to Treat the Most Frequent Diseases Encountered in Ambalabe Rural Community, Eastern Madagascar. *Journal of ethnobiology and ethnomedicine*, 11**,** 1-16.

Sanou, H & Lamien, N 2011. Vitellaria Paradoxa Shea: Conservation and Sustainable Use of Genetic Resources of Food Tree Species in Sub-Saharan Africa. *Safergen***,** 2-4.

Sofowora, A, Ogunbodede, E & Onayade, A 2013. The Role and Place of Medicinal Plants in the Strategies for Disease Prevention. *Afr J Tradit Complement Altern Med*, 10**,** 210-29.

Song, S, Lee, H, Jin, Y, Ha, YM, Bae, S, Chung, HY & Suh, H 2007. Syntheses of Hydroxy Substituted 2-Phenyl-Naphthalenes as Inhibitors of Tyrosinase. *Bioorganic & medicinal chemistry letters,* 17**,** 461-464.

Torrez, PP, Said, R, Quiroga, MM, Duarte, MR & Franca, FO 2014. Forest Pit Viper (Bothriopsis Bilineata Bilineata) Bite in the Brazilian Amazon with Acute Kidney Injury and Persistent Thrombocytopenia. *Toxicon,* 85**,** 27-30.

Vadivel, E & Gopalakrishnan, S 2011. Gc-Ms Analysis of Some Bioactive Constituents of Mussaenda Frondosa Linn. *International Journal of Pharma and Bio Sciences,* 2**,** 313-320.

Vickers, CE, Possell, M, Cojocariu, CI, Velikova, VB, Laothawornkitkul, J, Ryan, A, Mullineaux, PM & Nicholas Hewitt, C 2009. Isoprene Synthesis Protects Transgenic Tobacco Plants from Oxidative Stress. *Plant Cell Environ,* 32**,** 520-31.

Wang, GF, Shi, LP, Ren, YD, Liu, QF, Liu, HF, Zhang, RJ, Li, Z, Zhu, FH, He, PL, Tang, W, Tao, PZ, Li, C, Zhao, WM & Zuo, JP 2009. Anti-Hepatitis B Virus Activity of Chlorogenic Acid, Quinic Acid and Caffeic Acid in Vivo and in Vitro. *Antiviral Res,* 83**,** 186-90.

Warrell, DA 2010. Snake Bite. *Lancet,* 375**,** 77-88.

Williams, HF, Hayter, P, Ravishankar, D, Baines, A, Layfield, HJ, Croucher, L, Wark, C, Bicknell, AB, Trim, S & Vaiyapuri, S 2018. Impact of Naja Nigricollis Venom on the Production of Methaemoglobin. *Toxins (Basel),* 10**,** 539.

Yang, WD, Liu, YR, Liu, JS & Liu, Z 2008. [Inhibitory Effects and Chemical Basis of Eucalyptus Orelliana Wood Meals on the Growth of Alexandrium Tamarense]. *Huan Jing Ke Xue,* 29**,** 2296-301.

Yildiz, OG, Soyuer, S, Saraymen, R & Eroglu, C 2008. Protective Effects of Caffeic Acid Phenethyl Ester on Radiation Induced Lung Injury in Rats. *Clin Invest Med,* 31**,** E242-7.

Zhang, A, Sun, H, Wang, P, Han, Y & Wang, X 2012. Modern Analytical Techniques in Metabolomics Analysis. *Analyst,* 137**,** 293-300.