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GC-MS and NMR analysis of the bioactive compounds from the crude extracts of *Waltheria indica* and the histopathological changes induced in albino rats challenged with *Naja nigricollis* venom

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ABSTRACT

Objective: To develop a characteristic fingerprint of the different chemical compounds of plant by gas chromatography-mass spectrometer (GC-MS) and nuclear magnetic resonance (NMR) analysis and assess the effect of the crude extract on histological changes induced in *Naja nigricollis* (*N. nigricollis*) envenomed albino rats.

Methods: The coarsely powdered *Waltheria indica* (*W. indica*) whole plant material was extracted using 70% methanol. GC-MS analysis was carried out using a Shimadzu GC-MS QP 2010 plus system of gas chromatography interfaced to a mass spectrometer. The identification of components was based on National Institute of Standard and Technology-08 and Willey-8 libraries. Hydrogen-NMR spectra of the extracts was performed using Bruker Biospin Avance 400-MHz NMR spectrophotometer with a 5 mm broad inverse probe head, equipped with shielded z-gradient accessories and C-13 NMR hetero-nuclear single quantum correction was carried out using the Brucker's standard pulse library. The efficacy of the crude extract of *W. indica* whole plant was tested and the lesions induced in the liver, brain and kidney of albino rats challenged with *N. nigricollis* were analyzed by light microscopy.

Results: The identification of the bioactive compounds from *W. indica* by GC-MS analysis revealed 41 peaks. A total of 38 compounds were identified with majority having important pharmacological activities that included anti-microbial, anti-cancer, anti-oxidant anti-inflammatory, anti-allergenic, anti-ageing, anti-arthritic, larvicidal, anti-convulsant and herbicidal activities. C-13 and hydrogen-NMR analysis of *W. indica* elucidated key bioactive compounds in the whole plant that were consistent with the classes of bioactive compounds detected by GC-MS analysis. The efficacy of the crude extract of *W. indica* whole plant in ameliorating histopathological lesions induced in the liver, brain and kidney of albino rats challenged with *N. nigricollis* venom revealed the abrogation of tissue degeneration when compared with the experimental control group.

Conclusions: The present study identified an array of bioactive compounds present in *W. indica* and reported their ethno-botanical uses in the treatment of a wide range of medical exigencies and showed scientific evidence of the detoxification of *N. nigricollis* venom by *W. indica*.

1. Introduction

In developing countries of the world, traditional medicine still serves the need of primary health care for most of its population. Medicinal plants are important sources of medicines and presently constitute about 25% of pharmaceutical prescriptions[1]. According to the World Health Organization, the traditional medicine system continues to play an important role in healthcare system since over 80% of the population in the third world countries relies on traditional medicine[2].

Medicinal plants are rich sources of antioxidants and the bioactive compounds of most drugs are derived from plants sources[3,4]. Hence, for the purpose of exploiting medicinal plants as potential agents against different medical exigencies, it is imperative that the active principles are identified and the pharmacological properties are well documented considering that this will serve as an ethno-botanical database for further

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studies[5].

Waltheria indica (*W. indica*) is commonly known as sleepy morning, monkey bush, velvet-leaf, marsh-mallow, "hankufah" in Hausa, "kanakaloa" in Hawaii and "nallabenda" in India and is a plant growing in many regions of the world[6,7].

Existing reports revealed that *W. indica* has been reported to have the following activities: analgesic, anti-inflammatory, anti-bacterial, anti-fungal, anti-diarrhoeal, anti-malarial, anti-viral, anti-convulsant, anti-anaemic and anti-oxidant activities. The pharmacological studies performed on *W. indica* revealed therapeutic potential in the treatment of infectious diseases[6].

Extraction is the main step for the recovery and isolation of bioactive phytochemicals from plant materials, before component analysis[8]. Gas chromatography-mass spectrometer (GC-MS) is an approach used for the analysis of plant extracts and it can be an interesting tool for testing the amount of some active principles in the pharmaceutical or food industry[1]. It combines two analytical techniques to a single method of analyzing the mixtures of chemical compounds. Gas chromatography separates the components of the mixture and mass spectroscopy analyzes each of the components separately.

On the other hand, nuclear magnetic resonance (NMR) is an analytical method that provides the most comprehensive structural information, including stereochemical details[9]. NMR is the predominant profiling method because it is fast and simple, and the technique has been used as a major analytical tool for many applications in plant metabolomics[10].

In the current absence of any report on the GC-MS and direct NMR analysis of *W. indica*, we sort to profile the active metabolites of the plant using an advanced instrumentation approach which provides major technological platform for profiling the bioactive compounds in both plant and non-plant species with a view to build an ethno-botanical database for this plant which can be explored in the design of counter-measures against different diseases of medical relevance.

2. Materials and methods

2.1. Collection and identification of plant material

The plant, *W. indica* was obtained from wild in Zaria, Kaduna State, in Northern Nigeria and identified by Mr. U.S. Gala at the Herbarium Unit of the Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria and the plant had been documented with the voucher number 7353.

2.2. Extract preparation (hydro-alcoholic extraction)

The fresh whole plant material of *W. indica* was collected and shade-dried at room temperature. The coarsely powdered plant material (118.68 g) was extracted in 1 200 mL of 70 % methanol (BDH Chemicals Ltd Poole England 86542) in an orbital shaker at room temperature for 72 h. The extract was filtered with Whatman No. 1 filter paper and the filtrate was evaporated to dryness in crucibles using a temperature-regulated water bath (H1021. Cr42II. Grant Instrument, Cambridge Ltd) pre-set at 50 ˚C. The crystals were weighed and the crystalline extract was preserved at 4 ˚C in an airtight container for further use.

2.3. GC-MS analysis of W. indica

GC-MS analysis was carried out using a Shimadzu GC-MS QP 2010 plus system of gas chromatography interfaced to a mass spectrometer, comprising of an AOC-20i+s and an RTx-5 Sil MS column (30 m × 0.25 mm id × 0.25 film thickness).

The operating conditions of the column were as follows: oven temperature was programmed from 80–250 ˚C at 10 ˚C/min with hold time of 3 min and from 250–280 ˚C at 15 ˚C/min with hold time of 5 min, and the final temperature was set for 18 min. The injector temperature was maintained at 260 ˚C and the volume of injected sample was 8 µL; pressure was 85.3 kPa, total flow was 16.30 mL/min, column flow was 1.21 mL/min, linear velocity was 40.5 cm/sec, purge flow was 3.0 mL/min, split ratio was 10.0; ion source temperature was 230 ˚C, scan mass range of *m/z* was 40–600 and interface line temperature was 270 ˚C with scan interval of 0.50 sec and the total gas chromatograph running time was 44.98 min.

2.4. Identification of bioactive compounds

The identification of components was based on National Institute of Standard and Technology-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. The results obtained were tabulated and a web-based search for the pharmacological properties of the bioactive compounds identified by GC-MS analysis was carried out.

2.5. NMR analysis of W. indica

Hydrogen-NMR spectra of the extracts was performed using Bruker Biospin Avance 400-MHz NMR spectrophotometer with a 5 mm broad inverse probe head, equipped with shielded z-gradient accessories. Hydrogen-NMR spectral analysis of the extract was carried out using one-pulse sequence by dissolving the extract in 500 µL of deuterated methanol in 5 mm NMR tubes. A re-usable, sealed capillary tube containing 30 µL of 0.375% of tri-sodium phosphate in deuterium oxide was inserted into the NMR tube before recording the spectra. Tri-sodium phosphate served as chemical shift reference as well as internal standard for quantitative estimation. C-13 NMR heteronuclear single quantum correction was carried out using the Brucker's standard pulse library.

2.6. Haematoxylin and eosin staining of tissues

2.6.1. Animal source

Albino rats weighing 151–250 g were obtained from the Federal University of Lokoja, Kogi State, Nigeria. The animals were acclimatized for 2 weeks at the animal house facility situated at the Department of Biochemistry, Kogi State University, Anyigba, Nigeria. The guide for the care and use of laboratory animals, 1996 of the Institute of Laboratory Animal Research Commission on life Science, National Research Council was duly followed.

2.6.2. Venom source

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

2.6.3. Administration of N. nigricollis venom and treatment with W. indica

The venom was pre-incubated with the extract in an equal ratio (1:1) at a calculated dosage of 8 mg/kg body weight for venom and extract respectively. Intramuscular administration of the mixture to Group 1 rats was done 30 min after the pre-incubation step. The experimental control groups of rats (Groups 2 and 3) were administered the venom alone, and extract alone respectively. At 4 h post-envenomation, the liver, kidney and brain tissues were excised from each group. 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, absolute xylene for 4 days and embedded in paraffin. Sections were cut at 3.5 μm, stained with hematoxylin and eosin and analyzed by light microscopy.

3. Results

3.1. GC-MS analysis

The GC-MS analysis of *W. indica* indicated the presence of 41 phytochemical constituents. Comparison of the mass spectra of bioactive constituents with internal standards of the National Institute of Standard and Technology and Willey libraries, 38 out of the 41 bioactive compounds were identified. The active principles with their respective retention time, molecular formula, molecular weight and concentration (peak area, %) were presented in Table 1. The spectra of some key compounds with

Table 1
GC-MS spectral analysis of the extract of *W. indica*.

Sample No.	Names of compounds	MF	Compound nature	MW	RT (min)	Area (%)
1	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	Flavonoid	144	7.251	5.51
2	Benzoic acid	$C_7H_6O_2$	Aromatic carboxylic acid	122	8.202	2.98
3	2,3-Dihydro-benzofuran	C_8H_8O	Heterocyclic compound	120	8.501	10.24
4	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	Phenolic compound	150	9.736	2.02
5	6-hexadecen-4-yne (E)	$C_{16}H_{28}$	Hydrocarbon	220	11.307	0.35
6	6-Isopropenyl-3-(methoxy)-3-methyl-1-cyclohexene	$C_{12}H_{20}O_2$		196	11.758	0.33
7	Benzoic acid, 4-hydroxy, hydride	$C_7H_8N_2O_2$	Carboxylic acid	152	12.051	0.99
8	4-(6,6-Dimethylbicyclo(3,1,1)Hept-2-en-2-yl) 2B	$C_{13}H_{20}O$	Flavonoid	192	12.532	0.28
9	2(4h)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-Trimethyl-, (R)-	$C_{11}H_{16}O_2$		180	12.762	0.41
10	3-Tert-butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$		180	13.079	1.13
11	Bicyclo(3,2,1) octan-3-one, 6-(2-hydroxyethyl), endo	$C_{10}H_{16}O_2$	Benzene compound	168	13.575	0.20
12	Benzene, (1-butylheptyl).	$C_{17}H_{28}$		232	13.692	0.54
13	7-Oxabicyclo (4,1,0) heptane, 1-methyl-4-(2-methyloxiranyl)	$C_{10}H_{16}O_2$	Benzene compound	168	14.136	1.04
14	Benzene (1-methyldecyl)	$C_{17}H_{28}$	Flavonoid	232	14.483	0.86
15	Benzene, (1-hexyltetradecyl)	$C_{26}H_{46}$	Benzene compound	358	14.754	0.36
16	Tetradecanoic acid	$C_{14}H_{28}O_2$	Acid	228	15.207	0.42
17	Benzene, (1-methylnonadecyl)	$C_{26}H_{46}$		358	15.608	0.56
18	2,6,10-Trimethyl,1,4-ethylene-14-pentadecene	$C_{20}H_{38}$	Alkene	278	15.874	1.67
19	Cyclopropanenonanoic acid,2-(2-butylcyclopropyl) methyl	$C_{21}H_{38}O_2$	Carboxylic acid	322	16.322	0.64
20	Benzoic acid, 2-hydroxy, phenylmethyl ester	$C_{14}H_{12}O_3$	Ester	228	16.481	0.34
21	4-Oxazolecarboxylic acid,4,5-dihydro-2-phenyl-1-methyl ethyl ester	$C_{13}H_{15}NO_3$	Ester	233	18.240	1.68
22	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	Linoleic acid ester	294	18.444	1.24
23	9,12,15-Octadecatrienoic acid, methyl ester (ZZZ)	$C_{19}H_{32}O_2$	Linoleic acid ester	292	18.517	1.54
24	2-Hexadecen-1-ol, 3, 7, 11, 15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}O$	Terpene alcohol	296	18.639	4.41
25	9,12-Octadecadien-1-ol	$C_{18}H_{34}O$	Alcoholic compound	266	19.040	18.47
26	Octadecanoic acid	$C_{18}H_{36}O_2$	Stearic acid ester	284	19.217	1.75
27	1-Chlorooctadecane	$C_{18}H_{37}Cl$	Halo- alkane	288	19,996	0.36
28	1-Methyl-3-Phenyl-2-benzamidopropionate	$C_{17}H_{17}NO_3$		283	20.384	0.48
29	Benzene, 1-cyclopropyl-2-nitro-	$C_9H_9NO_2$		163	20.820	0.45
30	Cyclopropan, 1-(2-ethyl-2-propenyl-1-(2-methoxycarbonyl-trans-ethenyl)-	$C_{12}H_{18}O_2$		194	21.597	5.41
31	1H-purin-6-amine,[(4-fluorophenyl)methyl	$C_{12}H_{10}FN_5$		243	23.111	0.19
32	Cyclooctanecarboxilic acid,5-nitro-2-oxo	$C_{10}H_{15}NO_5$	Carboxilic acid	229	23.865	0.40
33	Cyclopropanecarboxylic acid, 2-methyl-2-(4-methyl-3 pentenyl)-,trans-(.+-.)-	$C_{11}H_{18}O_2$		182	24.222	6.54
34	α-Bisabolol	$C_{15}H_{26}O$	Sesquiterpene alcohol	222	24.775	0.52
35	3,6-Dibutyl-2H-azepine-2,5 (6H)-dione	$C_{14}H_{21}NO_3$	Alkaloid	235	25.784	0.08
36	2-Piperidinone, N-(4-bromo-n-butyl)	C_9H1_6BrNO	Alkaloid	233	27.439	0.17
37	2-Piperidinone, N-(4-bromo-n-butyl)	$C_9H_{16}BrNO$	Alkaloid	233	27.684	0.13
38	4-Tert-butylcatechol, bis (trifluoroacetate)	$C_{14}H_{12}F_6O_4$		358	30.975	0.21

MF: Molecular formula; MW: Molecular weight; RT: Retention time.

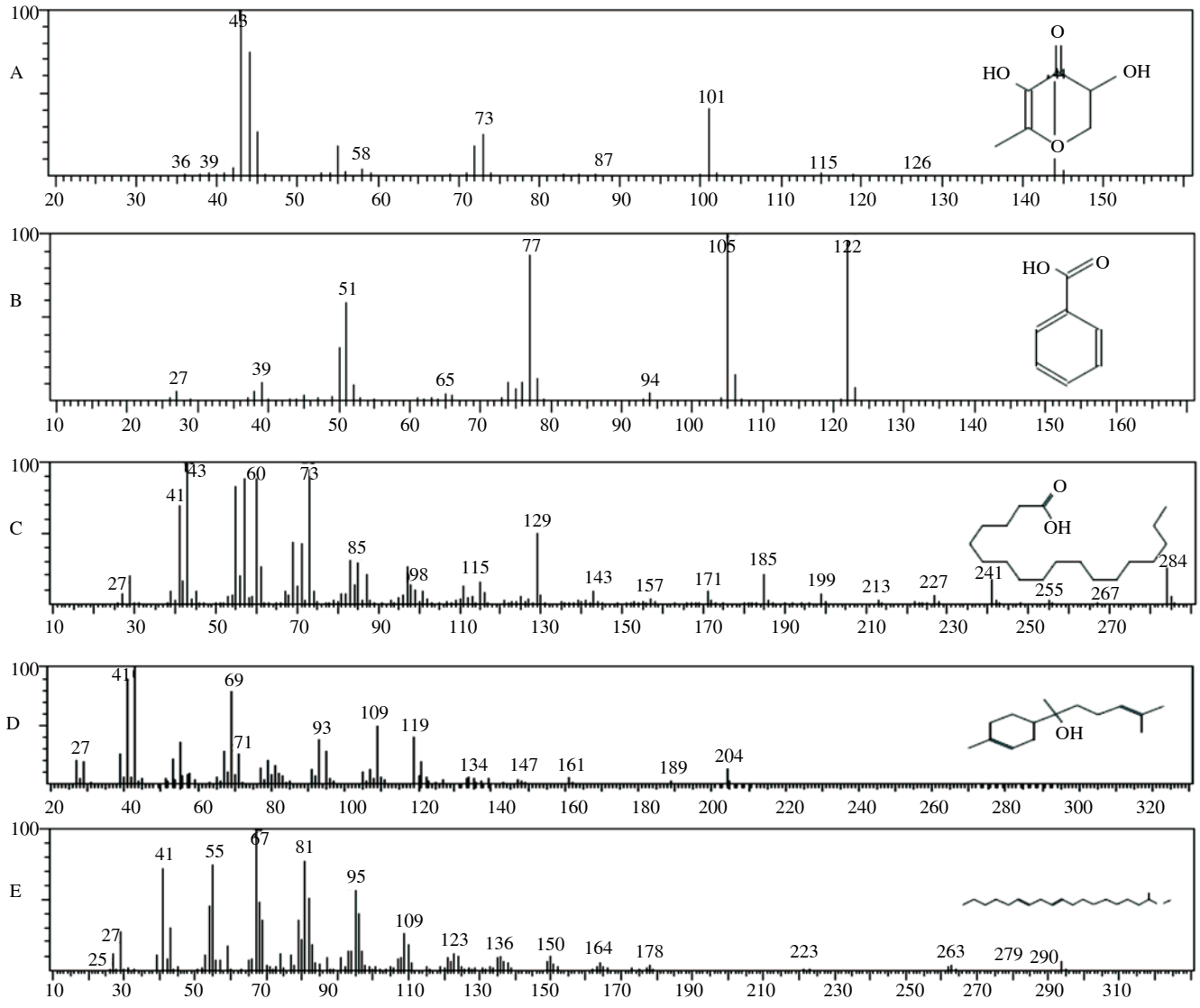

Figure 1. A: Mass spectrum of 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one; B: Mass spectrum of benzoic acid; C: Mass spectrum of octadecanoic acid; D: Mass spectrum of α-bisabolol; E: Mass spectrum of 9, 12-octadecadienoic acid, methyl ester.

biological activities that were identified from *W. indica* were presented in Figure 1. The biological activities of the various bioactive components identified in the crude extract of *W. indica* were discussed accordingly.

3.2. NMR analysis

Hydrogen-NMR-based analysis was sufficient to generate data from a sample within a relatively short time. Hydrogen-NMR and C13 NMR spectra of *W. indica* revealed several signals as shown in Figures 2 and 3 respectively. The bioactive compounds in the crude extract were individually characterized by their respective chemical shifts values as shown in the spectra below.

3.3. Histopathological analysis

Photomicrographs for the analysis on the histological changes observed in the different tissues excised from the respective groups of albino rats were shown in Figures 4–6.

A comparative histological analysis of livers excised from the respective groups of experimental rats (extract alone, venom alone, venom and extract) revealed that there was convincing evidence of darkening of nuclear material, focal necrosis, enlarged sinusoids and vascular congestion in the tissues of rats that received the extract alone (Figure 4A). But in the group that received the venom alone (Figure 5A), we observed severe necrosis of hepatocytes, breakdown of sinusioidal wall integrity and sinusoidal congestion. However, in the group of rats that received the extract and venom in combination (Figure 6A), the hepatocytes seemed to be undergoing a delayed-type degeneration exemplified by gradual focal necrosis, vascular and sinusoidal congestion.

Similarly, we comparatively analysed the histopathological data for the brain tissues excised from the respective groups of rats and we did not observe any discernable histological changes (Figure 5B and Figure 6B) except for the significant macrophage infiltration in the tissues of rats that received the extract alone (Figure 4B).

When we compared the histo-architecture of the kidney tissues excised from the respective experimental groups of rats, we observed mild cellular vacuolation, tubular necrosis and macrophagic infiltration in the tissues of rats that received the

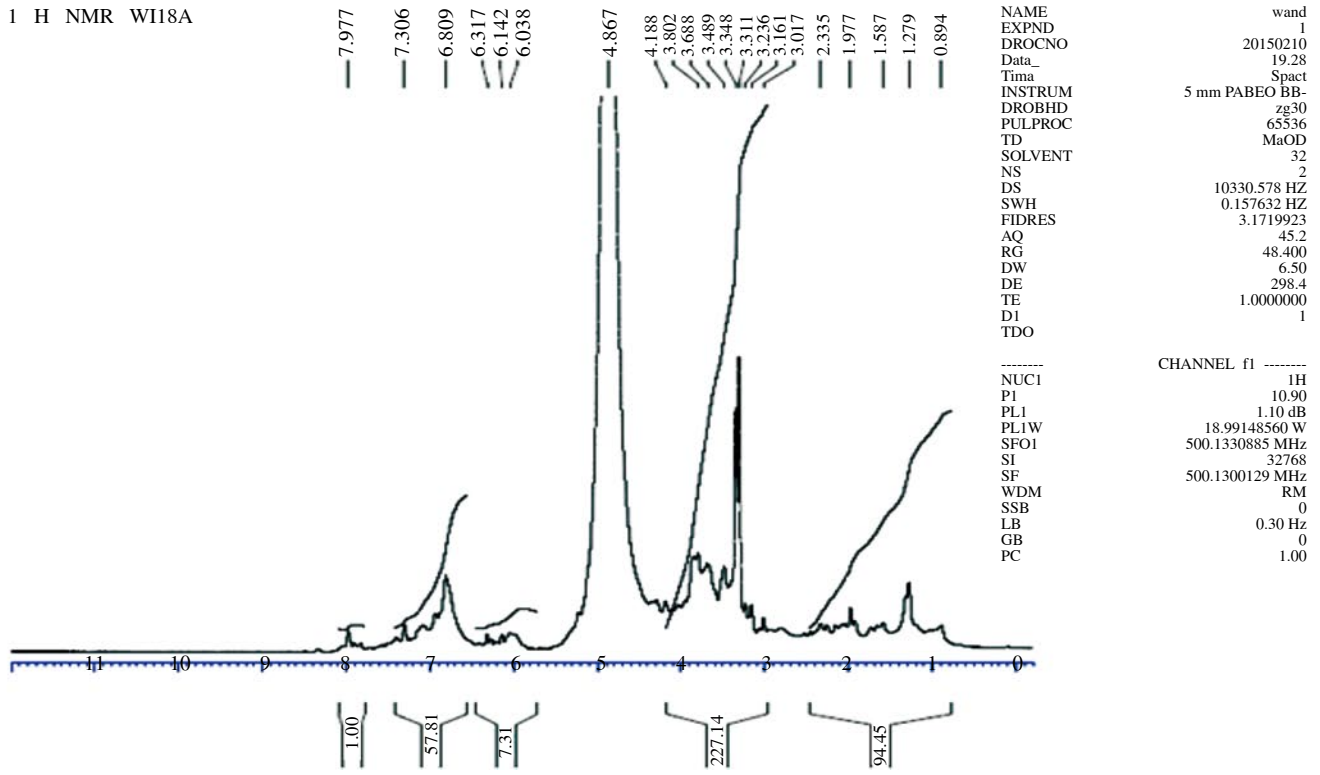

Figure 2. Hydrogen-NMR spectrum of *W. indica*.

The upper values represented the chemical shifts which indicated the bioactive compounds and corresponded to the lower values which was in part per million (ppm).

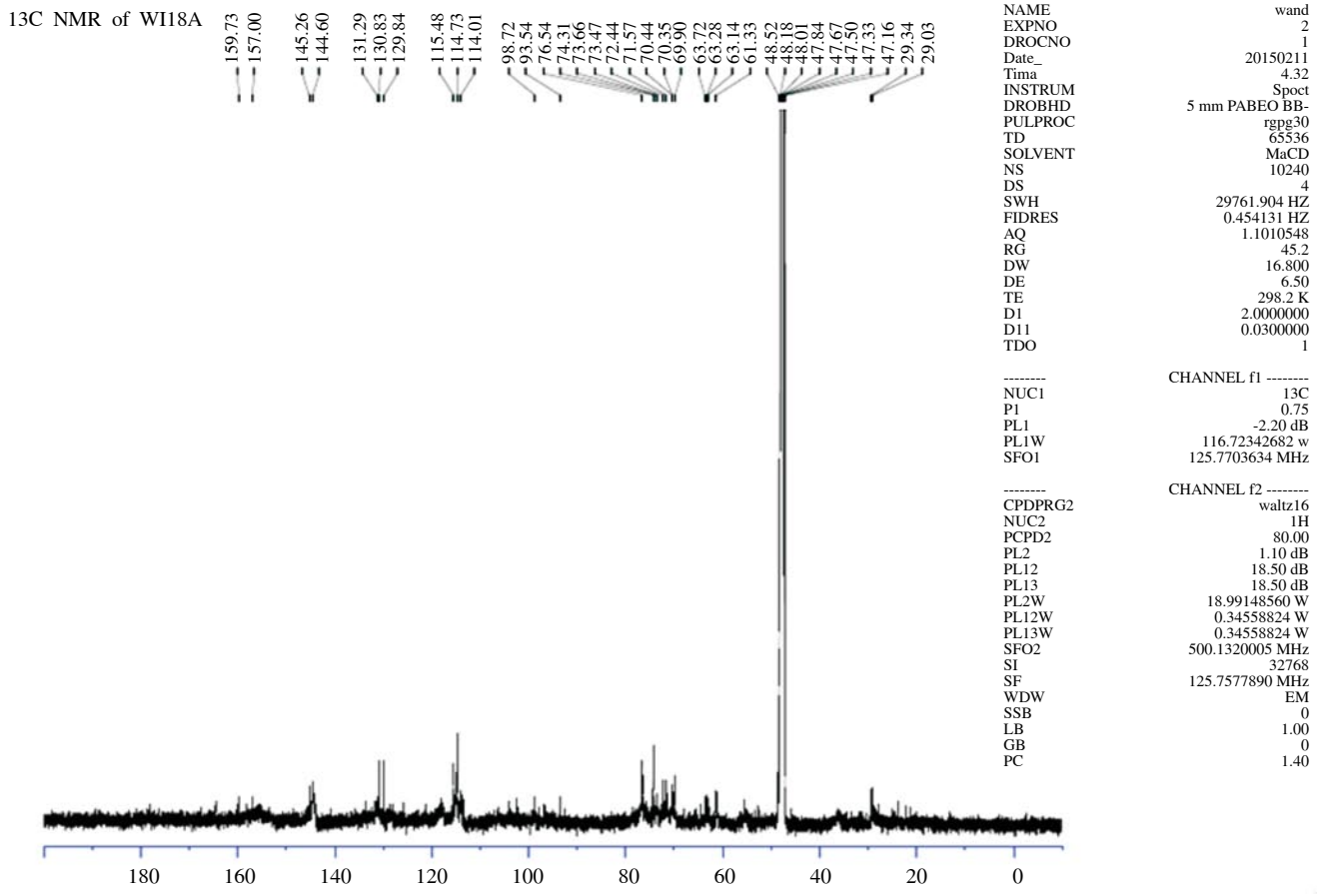

Figure 3. C-13 NMR spectra of *W. indica*.

The upper values represented the chemical shifts which indicated the bioactive compounds and corresponded to the lower values which was in part per million (ppm).

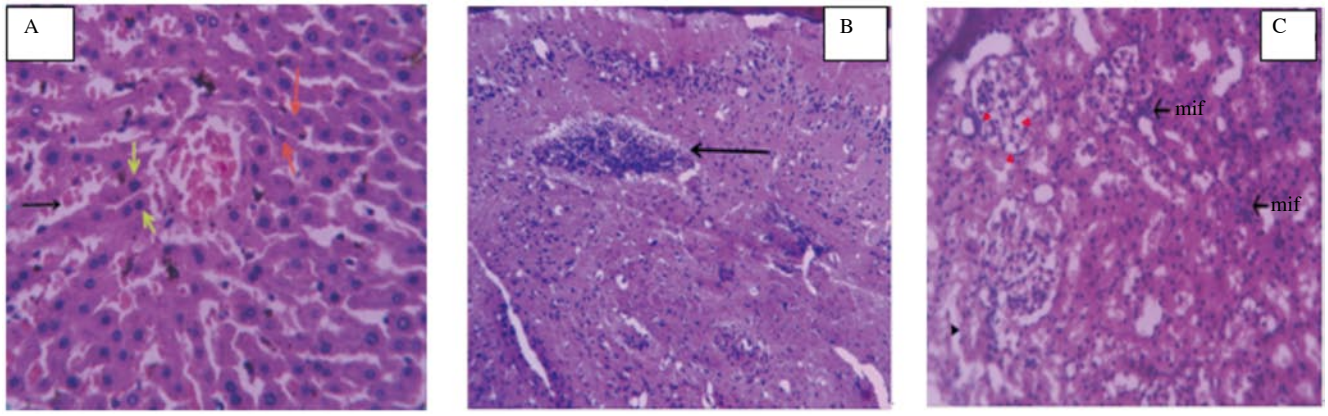

Figure 4. Histopathological lesions observed in albino rats in Group 3, administered the extract alone.
A: Section of liver tissue (×400) [an evidence of darkening of nuclear material (green arrow), focal necrosis of some hepatocytes (red arrows); enlarged sinusoids (black arrow) and central vein congestion]; B: Section of cerebral cortex (×100) [an evidence of significant macrophage infiltration (black arrow)]; C: Section of kidney (×250) [an evidence of cellular vacuolation (red arrow), tubular necrosis (black arrow) and mif: Mild macrophagic infiltration].

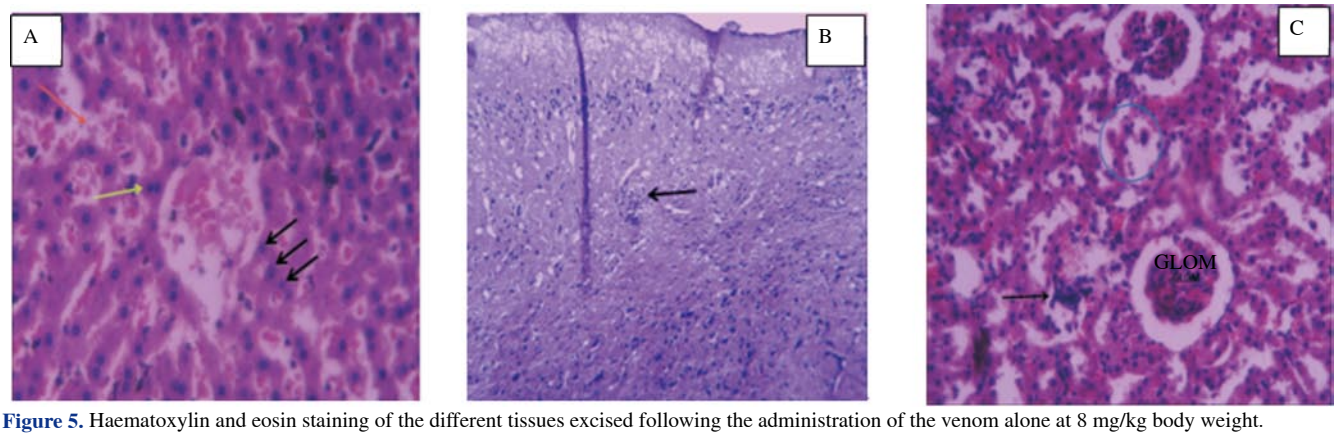

Figure 5. Haematoxylin and eosin staining of the different tissues excised following the administration of the venom alone at 8 mg/kg body weight.
D: Section of liver tissue (×400) [an evidence of severe necrosis of hepatocytes (black arrow), breakdown of sinusioidal wall integrity and sinusoidal congestion (blue arrow)]; E: Section of cerebral cortex (brain) [cerebral histo-architecture was preserved, thus no significant histopathological changes were seen]; F: Section of renal cortex kidney (×250) [an area of tubular necrosis (blue circle), mild macrophage infiltration (black arrow) and intact glomerulus (GLOM).

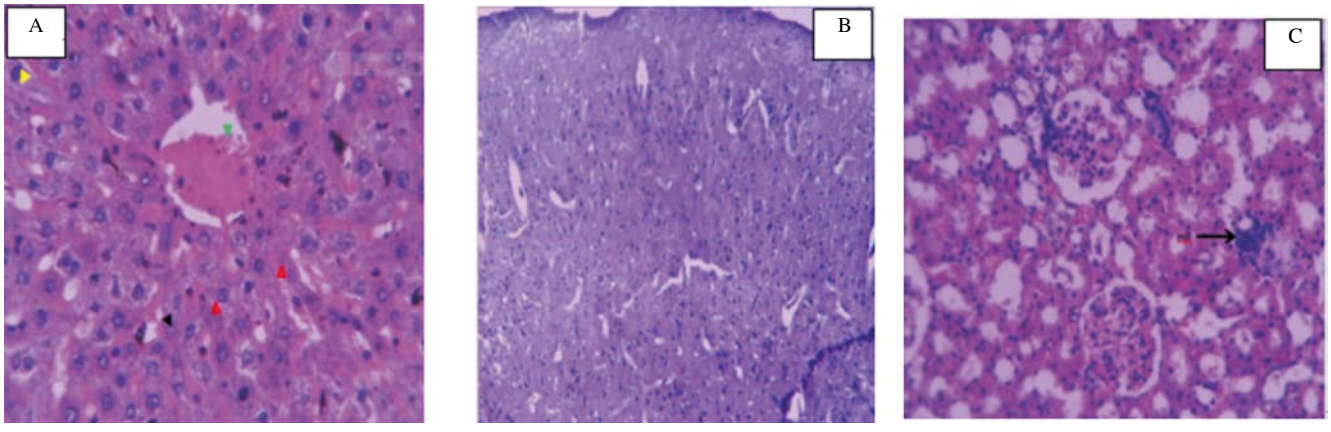

Figure 6. Haematoxylin and eosin staining of the different tissues excised following the administration of 1:1 ratio of *N. Nigricollis* venom and *W. indica* extracts.
G: Section of liver tissue (×400) [some hepatocytes were undergoing degeneration (black arrow), a focal necrosis of some hepatocytes (red arrow), a vascular and sinusoidal congestion (green and yellow arrows) (FP: formalin pigment artifact)]; H: Section of cerebral cortex (×100) [no significant histopathological changes were seen]; I: Section of kidney (×250) [an evidence of a preserved renal cortex histo-architecture].

extract alone (Figure 4C). But in the group of rats that received the venom alone, severe tubular necrosis and mild macrophage infiltration were observed (Figure 5C). However, in the group of rats that received the extract and the venom in combination, there was a distinct evidence of a preserved histo-architecture of the renal cortex and intact glomerulus, even though we observed mild macrophage infiltration (Figure 6C).

4. Discussion

Orthodox medicine has been practised for many years and this has emphasized the need for the discovery and modernization of various therapeutic agents. Extraction, analysis and characterization of medicinal plant are major advancements in the search for counter-measures against pathogens that poses threats to humans and other animals. Therefore,

the identification and characterization of bioactive compounds from medicinal plants improves our current understanding of plant toxicity, the mechanism of action and uptake of the test compound to physiologically active level that elicits therapeutic effects[11]. On account of our GC-MS analysis of *W. indica*, we have presented the fingerprints of the various bioactive compounds and determined their biological activities based on web-based search.

W. indica contains tetradecanoic acid (myristic acid) which is known to have anti-bacterial and anti-inflammatory properties[12]. Other biological activities of the aforementioned compound include larvicidal, anti-oxidant, anti-cancer, hyper-cholesterolemic, nematicide, fragrance, opacifying agent, surfactant, cleansing agent, emulsifier and in cosmetic[13].

Furthermore, octadecadienoic acid also present in the plant extract has the property of anti-inflammatory, anti-oxidant, anti-microbial, hypo-cholesterolemic and anti-arthritic activities that have been previously reported[1,14,15,16]. Similarly, 2, 3-dihydro-benzofuran contained in the GC-MS fingerprint has been noted for fungicidal, pro-oxidant, anti-angiogenic activities and is a potent inhibitor of acetyl cholinesterase activity which could be exploited for the development of anti-venin[17].

Interestingly, our GC-MS fingerprints revealed the presence of sesquiterpenes which have been identified as the active constituents present in several medicinal plants with a wide range of biological properties including anti-infective, anti-oxidant, anti-inflammatory, anti-cancer and anti-cholinesterase activities[13]. Furthermore, sesquiterpenes are also associated with important biological and physiological functions such as pheromone interactions, anti-feedants, phyto-alexins, *etc*.

α-Bisabolol, a monocyclic sesquiterpene alcohol, is one of the active principles identified in the GC-MS analysis of *W. indica*. It has been widely used as an ingredient in dermatological and cosmetic formulations such as after shave creams, hand- and body-lotions, deodorants, lip sticks, sun-care and after-sun products, baby care products and sport creams[18]. It may be a preferred active ingredient for protection against the recurring stresses of the environment on the skin.

The main application of α-bisabolol in the pharmaceutical sector is related to its anti-inflammatory, anti-spasmodic, anti-allergic, drug permeation and vermifuge properties. Bisabolol and bisabolol rich-oil have also demonstrated the capacity to enhance the percutaneous absorption of certain molecules and may prove to be an important ingredient for cosmetic and skin care products in the future[18,19].

We detected the presence of saponins in the plant extract and Kang *et al*., have recently reported the reactive oxygen-scavenging activity of saponins in garlic[20]. Saponins are also known to have antibiotic activity and many anti-tumour drug preparations used for chemotherapeutic management of various types of cancer contain saponins in their chemical formulations[21-25]. Even more, previous reports exist on the claims of the use of *W. indica* for experiential treatment of snakebites[6]. In line with this, Dileep *et al*. demonstrated that catechol and its derivatives bind competitively to phospholipase A_2, an enzyme found in the venoms of snakes, scorpions and insects[25].

Profiling of the bioactive principles of *W. indica* whole plant by hydrogen-NMR and C-13 NMR revealed the presence of different saturated fatty acids at signal ɗ 1.587 and ɗ 2.335. Proton-NMR signal was detected at ɗ 0.809 and C-13 NMR signals at 63.14, 69.90 respectively that corresponds to the chemical shift values of withaferin-A, which is reported previously to have anti-inflammatory activity[26]. Additionally, withaferin-A is also a metabolite that has been reported to have anti-tumour properties and this corroborated earlier reports on the anti-cancer activity of withaferin-A[26,27]. Inositol-signal at ɗ 0.894 can combine with phosphate to form inositol hexa-phosphate which functions mainly in the regulation of cell proliferation and hence other cellular reactions[28].

Proton-NMR analysis revealed chemical shift values of ɗ 6.038–6.809 and C-13 NMR chemical shift values of ɗ 129.84 that corresponds to carotenoids. Based on epidemiological studies, a direct correlation is suggested between higher dietary intake and tissue concentration of carotenoids and lower risk of chronic diseases[29,30]. Lycopene is a derivative of carotenoids and has been shown to prevent the risk of cardiovascular diseases and certain cancers[29,30]. Also, lycopene has been reported to have potent antioxidant properties and preventive role in oxidative stress-mediated chronic diseases and hence, having a pharmacological effect on pathogenesis of the skeletal system including prostrate cancer[31].

β-Sistosterol is a derivative of sterols signalling at ɗ 0.894 and sterols have been noted to play an important role in lowering plasma cholesterol level[32]. Reduced plasma cholesterol consequently lowers serum cholesterol concentration despite the compensatory synthesis of cholesterol that occurs in the liver and other tissues are secreted into the blood stream. Despite the limitations for the analysis of crude extracts by NMR due to overlapping signals, we observed consistencies in our GC-MS fingerprints and NMR spectra, base on the detection of phyto-compounds and/or derivatives with similar pharmacological activities.

Organ dysfunction has been ascribed to the effects of necrotic and cytotoxic components of snake venoms (cyto/cardio-toxins, necrotic and hemorrhagic factors/toxins and several enzymes) including a major class of enzyme called phospholipases[33]. Histological, immuno-histochemical and biochemical fluctuations triggered by the venom of *N. nigricollis* and *Naja haje* have been evaluated previously on rodents[34]. These changes may be as a result of leakage of membrane caused by the action of phospolipases and other enzymes present in the venom.

We studied the pathology of *N. nigricollis* envenomation in albino rats and histological examination of the selected organs revealed drastic degeneration of the kidneys that was ameliorated when rats were treated with *W. indica* extract. Our observation is substantiated by a report indicating that renal dysfunction is very common following viper bites and this is more devastating with elevated levels of haemotoxins and vasculotoxins in venom[35].

Herein, we identified an array of bioactive compounds present in *W. indica* and reported their ethno-botanical uses in the treatment of a wide range of medical exigencies. This could be exploited for precise drug targeting against various pathological conditions. We have also shown scientific evidence of *N. nigricollis* venom detoxification by *W. indica*. Therefore, *W. indica* is recommended as a plant of phyto-pharmaceutical importance on account of the abundance level of major phyto-compounds that can be utilized by drug-designers following appropriate isolation and characterization procedures for the active principles.

Conflict of interest statement

We declare that we have no conflict of interest.

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