

FTIR and GC-MS Analyses of Phytochemicals from Methanol Leaf Extract of *Cissus Multistriata* and Physiological Changes Induced in Male Rats Exposed to *Naja Nigricollis* Venom

James Omale^{*}, Umar Farouq Idris, Ojodale Adejoh, Abu Paul

Department of Biochemistry, Kogi State University, Anyigba, Nigeria

Email address:

james.omale123@yahoo.com (J. Omale)

^{*}Corresponding author

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Abstract: The present study sought to develop a blueprint for the identification of phyto-chemicals present in the methanol leaves extract of *Cissus multistriata* by Fourier transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometer (GC-MS) and test its antivenom activities against *Naja nigricollis* venom in male rats. Powdered leaves of *C. multistriata* were extracted using 50% methanol. FTIR analysis was carried out using SHIMADZU FTIR-8400S while GC-MS analysis was carried out using a SHIMADZU Japan GCMS-QP2010 PLUS mass spectrometer coupled to a SHIMADZU gas chromatograph. The antivenom property of the plant was tested by intraperitoneal (i. p.) injection of the extract at a dose of 100 mg/kg body weight of rats. Venom was administered i. p. at a dose of 1 mg/kg body weight of rats after 30 minutes. The IR spectrum suggested the presence of methyl, hydroxyl and unsaturated carbon-carbon double bonds in the bioactive compounds of the leaf extract. Two likely compounds as derived from the GC-MS chart are 2-(2-hydroxypropyl)-1,4-benzenediol, 2, 6, 8-Trimethylbicyclo[4, 2, 0]oct-2-ene-1,8-diol. Envenomation with *N. nigricollis* venom led to significant decreases in the levels of total protein and albumin with concomitant increase in creatinine, urea and uric acid levels. The plasma activities of alkaline phosphatase (ALP), aspartate amino transaminase (AST), and alkaline amino transferase (ALT) were significantly increased as well as the serum levels of sodium, potassium and chloride in rats challenged with venom compared with control rats. The above results indicate that *C. multistriata* contains numerous bioactive compounds and can neutralize *N. nigricollis* snake venom.

Keywords: Bioactive Compounds, Electrolytes, Fourier Transform Infrared Spectroscopy, Gas Chromatography-Mass Spectrometer, Liver Function Tests, Plasma, Snake Venom

1. Introduction

Snakebite is a major public health challenge and made worse by the fact that there are no adequate records of the actual incidence rates. Data presented to the World Health Organization (WHO) by a project director from the Nigerian Ministry of Health in 2007 showed that globally about 1 million cases of snakebite situations are recorded out of which about 20, 000 deaths result [1]. Kasturiratne and coworkers (2010) [2] estimated the global cases of snakebite at 5, 000, 000. One fifth of snakebite cases in West Africa are

accounted for by Nigeria. Data from Nigerian health centres showed that 174 out of every 100, 000 admissions are as a result of snakebite [3]. The number is not inclusive of unreported cases.

Conventionally, snakebite envenomation is treated with parenteral administration of horse or sheep-derived antivenoms to neutralize the toxins [4]. In third world countries, however, traditional medicine still constitutes an important component of their primary healthcare system. 25% of pharmaceutical prescriptions are constituted by medicinal plants. This underscores the fact that medicinal

plants are an important source of medicines [5, 6]. WHO reported that the traditional medicine institution perpetually plays very significant role in the healthcare system. This is because about 80% of the people of the third world countries relied on traditional medicine [5].

Medicinal plants are known to be an important source of antioxidants and they are the source of active ingredients of most drugs derived from natural plant sources [5, 7, 8]. Thus, for the purpose of exploiting medicinal plants for their pharmacological potentials, it is expedient to identify and characterize the bioactive compounds for proper documentation to serve as an ethno-botanical database for subsequent studies [5].

The main step for the recovery and isolation of bioactive compounds from plant materials is extraction [5]. Extraction is followed immediately by the analysis of the components of the plant extract. Gas chromatography-mass spectrometry (GC-MS) is a technique used in analyzing plant extracts and it is an interesting tool for testing the amount of bioactive chemicals in the pharmaceutical or food industry [5]. GC-MS combines two analytical techniques in a single method of analyzing the mixtures of chemical compounds. Gas chromatography separates the components of the mixture while mass spectroscopy analyzes each of the components separately.

On the other hand, Fourier Transform Infrared Spectrophotometry (FTIR) is an analytical technique based on absorption in the infrared region which results in changes in vibrational and rotational status of the molecules. The absorption frequency depends on the vibrational frequency of the molecules, whereas the absorption intensity depends on how effectively the infrared photon energy can be transferred to the molecule, and this depends on the change in the dipole moment that occurs as a result of molecular vibration. As a consequence, a molecule will absorb infrared light only if the absorption causes a change in the dipole moment. Thus, all compounds except for elemental diatomic gases such as N₂, H₂ and O₂, have infrared spectra and most components present in a flue gas can be analyzed by their characteristic infrared absorption. FTIR is used in this study to quantify the several components absorbing in the mid-infrared region (400-5000 cm⁻¹), present in the plant extract [9].

Cissus is a plant with over 350 different species found in the tropical and subtropical countries like Togo, Nigeria, Sudan, East Africa, Belgian-Congo and Angola [10]. Existing reports revealed that the plant has antimicrobial [11], analgesic, and has been used as an expectorant [10]. However, no scientific reports were found for its anti-snake venom potential. Thus, the aim of the present study was to investigate the ability of *Cissus multistriata* to confer some level of resistance against snake envenomation especially *Naja nigricollis*.

2. Materials and Methods

2.1. Chemicals and Reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl) was obtained from

Sigma (Germany). Chloroform and methanol were obtained from British Drug House (BDH). Assay kits for liver function tests were obtained from Randox Limited while creatinine and uric acid reagents were obtained from Quimica clinica. All other chemicals used in this study were of the highest grade and purity manufactured by Sigma-Aldrich (St. Louis, MO, USA) and British Drug Houses (Dorset, Poole, UK).

2.2. Animal Model

Thirty one healthy male albino rats weighing between 80 g and 120 g at the time of study were purchased from the breeding unit of the biochemistry department laboratory, Kogi State University, Anyigba, Nigeria for the study. The rats were acclimatized for a period of one week after purchase. They were housed 6 rats per cage in plastic cages placed in a well-ventilated animal house, provided with rat pellets purchased from vita feeds, Anyigba and provided water *ad libitum*. They were subjected to natural photoperiod of 12-hour light: dark cycle, for the period of acclimatization and experiments.

2.3. Experimental Design and Administration of Venom and *Cissus Multistriata*

The animals were randomly divided into five groups of at least six animals:

Animals in group I received drinking water and served as control. Group II rats received normal saline only. Group III rats received intraperitoneal injection of 1 mg/kg body weight of venom only. Group IV animals received intraperitoneal injection of 100 mg/kg body weight of the plant extract while group V animals were co-administered with venom and extract.

Group V animals were injected with the same dose (1 mg/kg of body weight) of *Naja nigricollis* venom 30 minutes after the plant extract was administered i. p. at 100 mg/kg body weight. All animals were observed over a period of the experiment. All experimental animals and control rats were sacrificed 2 hours after administration.

2.4. Source of Snake Venom and Collection of Plant Material

The crude venom was obtained from the *Naja nigricollis* snakes kept in large tanks at the serpentarium at the department of zoology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Heat was provided by a 100 W lamp for a daily period of 9 hours. Water was always available and the snakes fed on laboratory-bred mice every 10 - 14 days. Venom was milked from adult snakes, dried and reconstituted in saline solution prior to envenomation.

Plant material was collected from its natural habitat on Kogi State University Campus, Anyigba. The leaves were rinsed properly in water and air-dried at room temperature for four weeks. The dried leaves were then pulverized into powder using an electric blender and stored in polythene bag prior to use.

2.5. Preparation of Extract

200 grams of the powdered plant material was extracted in two litres of distilled water and left to soak for 48 hours. The solution was filtered using a 100% regular diameter sieve followed by a Fisher vacuum pump-enhanced filtration. The filtrate obtained was heated using a steam bath until a dry concentrate was obtained. The weight of the solid concentrate was determined and the percentage yield calculated.

2.6. Partial Purification of Plant Extract

2.6.1. Fragmentation of Concentrate Using Column Chromatography

Column chromatographical analysis of the plant extract was performed using the method described by Still *et al.*, (1978) [12]. Briefly, a clean glass chromatography column fitted with a fritted disk at the bottom and a stop cork at the outlet with separating funnel of 200 ml was aligned in a vertical position with the aid of a retort stand. Using a 500 ml beaker, slurry of silica gel and eluting solvent (50% methanol) was prepared. The column was packed slowly and evenly to about two-third full with silica gel slurry. The stop cork was opened to allow the solvent to drain into the beaker. The side of the chromatographic tube was gently tapped to make the silica gel compact, and after draining, the cork was closed. 3.7 g of the plant concentrate was dissolved in 5 ml of 50% methanol. 2 g of silica gel was then added and mixed thoroughly. This was gently poured along the walls of the column to prevent bubbles and turbulence. 50 ml of eluting solvent (50% methanol) was poured into the column gently. The cork was opened. From the solution obtained in the collecting glass jar, the solvent was evaporated leaving a paste, which was stored in a vial.

2.6.2. Further Purification Using TLC

The method described by Brett and Shixin (2010)[13] was adopted in carrying out the TLC analysis of the plant extract. Briefly, silica gel-pre-coated, already made thin layer plates obtained at G. S. O. and Company, Enugu, Nigeria were used. The sample was added to the plate in a process called "spotting." 1.0 mg of the sample was first made into solution by dissolving in 100 ml of 98% methanol. The solution was spotted on the TLC plate at the origin (2 cm from the bottom of the TLC plate) with a capillary tube. The prepared plate was then placed in the developing tank. The tank was then covered with a watch glass. This setup was left undisturbed on the bench to run until the solvent was about half a centimeter below the top of the plate. The spots were then visualized using iodine vapour in a desiccator. Retardation factor (Rf) values for the spots on the TLC plates after visualization were calculated using the formula:

$$R_f = \frac{\text{Distance moved by compound}}{\text{Distance moved by solvent front}} \quad (1)$$

2.6.3. Fourier Transform Infrared Spectroscopy of the 50% Methanol Extract

2 mg of the 50% methanol solid concentrate obtained from

the column chromatographic procedure was dissolved in methanol and made up to 100 ml. 10 μ l of the solution obtained was transferred into a silica cell. Another matched silica cell containing equal volume of only methanol was also prepared and both cells placed in the appropriate chambers in a Shimadzu FTIR-8400S Fourier Transform Infrared spectrophotometer which automatically showed the readings. The readings are presented in terms of transmittance and wavenumber (cm^{-1}).

2.6.4. GC-MS Analysis of the 50% Methanol Concentrate

The method described by Lisa *et al.*, (2008) [14] was adopted in carrying out the GC-MS analysis of the plant extract. Analyses were performed using a SHIMADZU Japan QP2010 PLUS mass spectrometer coupled to a SHIMADZU gas chromatograph. Prior to GC-MS, dry methanolic extracts were derivatized by adding 100 μ l of BSTFA at room temperature for 15 minutes (phenolic compounds TMS almost instantaneously under these conditions and no evidence of non-TMS derivatized phenolics were detected in the MS). Sample volumes of 1 μ l were injected into the gas chromatograph. Separation of the analytes was achieved using a SHIMADZU 5MS capillary column (30 m x 0.25 mm I. D., 0.25 μ m film thickness). Helium was used as carrier gas with a linear velocity of 46.3 cm/s. The GC injector temperature was 250°C; the transfer line temperature was held at 280°C. The mass spectrometer parameters were: start time of 3.00 min, end time of 37.00 min ACQ mode is scan, event time of 0.50 sec, scan speed of 1428; start m/z is 40.00 while the end m/z is 700.00 with a sample inlet unit GC. The MS program used was OFF.

2.7. Preparation of Crude Venom and Plant Extract Prior to Treatment

0.025 g of the crude venom was weighed and dissolved in 5 ml of normal saline. 0.25 g of the partially purified plant extract was weighed and dissolved in 10 ml normal saline. The standard sublethal dose for venom was 1 mg/kg body weight while that of the partially purified plant extract was 100 mg/kg body weight. Dosages were administered according to the individual body weight of the experimental animals.

2.8. Liver Function Tests and Enzyme Activity Assay

Plasma albumin and Urea nitrogen as well as serum potassium, chloride and sodium levels were measured using Teco Diagnostics standard assay kits; and the procedures according to the manufacturer's instructions. Plasma creatinine and uric acid levels were measured using Quimica Clinica standard assay kits; and the procedure according to the manufacturer's instructions.

Measurements of plasma alanine amino transferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) activities were carried out using RANDOX standard assay kits; and the procedure according to the manufacturer's instructions. Enzyme activities are expressed in U/l.

2.9. Electrolyte Tests

Electrolyte tests were carried out in the serum of experimental animals. Measurements of serum sodium, potassium and chloride were carried out using RANDOX standard assay kits; and the procedure according to the manufacturer's instructions. Serum concentrations of the electrolytes were given in mEq/L.

2.10. Measurement of Antioxidant Activity

Free radical scavenging activities of the plant extract were

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (2)$$

3. RESULTS

3.1. Weight of Extract

Table 1. Weight of Extract.

Sample	weight (g)
Pulverized plant	200
Extract after concentration	10.2

The weight of extract obtained upon pulverization and subsequent concentration is given in table 1. Percentage yield is 5.1.

3.2. Retention Values (Rf Values)

Table 2. Rf values of fifty percent methanol leaf extract of *Cissus multistriata* on TLC plate.

Bands	Rf Values
A	0.19
B	0.34
C	0.44
D	0.58Rf, retention values, TLC, thin layer chromatography

3.4. FTIR Analysis

Table 4. FTIR analysis result showing peaks from 50% methanol leaf extract of *Cissus multistriata*.

	Peak	intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	396.38	29.07	0.989	397.35	339.48	17.786	-0.21
2	466.79	17.757	0.306	472.58	464.86	5.74	0.041
3	1039.67	51.501	1.661	1082.1	934.54	40.122	0.621
4	1110.07	51.426	1.283	1171.79	1082.1	25.157	0.522
5	1415.8	47.774	0.156	1500.67	1411.94	27.478	0.104
6	1584.57	46.273	0.121	1601.93	1582.65	6.419	0.017
7	2130.45	56.849	0.007	2131.41	1964.56	40.23	0.12
8	3418.94	44.626	0.018	3523.1	3417.98	36.239	0.178
9	4006.28	63.467	0.032	4255.11	4005.32	42.273	-0.594
10	4355.41	69.565	0.603	4380.49	4255.11	18.998	0.086
11	4411.35	69.119	1.522	4503.93	4380.49	18.427	0.384
12	4700.68	70.647	0	4700.68	4654.39	6.959	0.001

3.5. GC-MS Chart

The IR analysis (table 4) showed absorption characteristics to O-H (stretch), -CH₃ (asymmetry), C=C (stretch). The IR analysis suggests that among the functional groups of the active compounds of fifty percent methanol leaf extract of *Cissus multistriata* are methyl, hydroxyl, and unsaturated carbon-carbon double bonds. The most likely corresponding compounds as derived from GC-MS chart (Fig. 1) are the compounds given in figure 2.

measured using the method described by Blois (1958) [14] with little modification. Briefly, a portion (1 ml) each of the different concentrations (1, 0.5, 0.25, 0.625, mg/ml) of the extract or standard (Quercetin) in test tubes were added 1 ml of 0.3 mM DPPH in methanol. The mixtures were vortexed and incubated in a dark chamber for 30 minutes after which the spectral readings were taken at 517 nm wavelength against a DPPH control containing only 1 ml of methanol in place of the extract. Percentage scavenging activity was calculated using the mathematical expression below:

3.3. Radical Scavenging Activity of Fifty Percent Methanol Leaf Extract of *Cissus Multistriata*

Table 3. DPPH radical-scavenging activity of fifty percent methanol leaf extract of *Cissus multistriata*.

Plant extract/standard	Concentration (mg/ml)	Scavenging activity (%)	IC ₅₀ (mg/ml)
Leaf Extract	1.000	80.50	0.479 ^a
	0.500	60.72	
	0.250	38.55	
	0.125	27.68	
	0.0625	9.88	
Quercetin	1.000	90.68	0.296 ^b
	0.500	78.60	
	0.250	56.10	
	0.125	41.62	
	0.0625	18.48	

^a: Linear equation: $y = 69.43x + 16.76$; ^b: Linear equation: $y = 69.25x + 29.48$. The antioxidant activities of the plant extract is shown in table 3. The free radical scavenging activity of the plant extract (0.479 mg/mL) is comparable with the activity of the standard quercetin used (0.296 mg/mL).

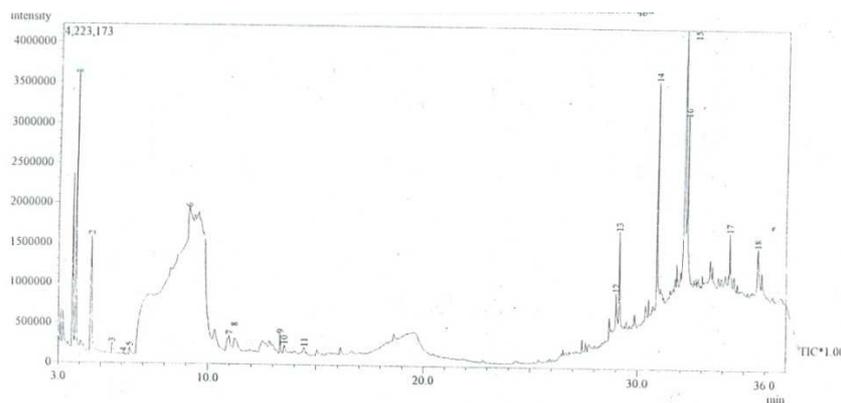


Figure 1. GC-MS chart of fifty percent methanol leaf extract of *Cissus multistriata*.

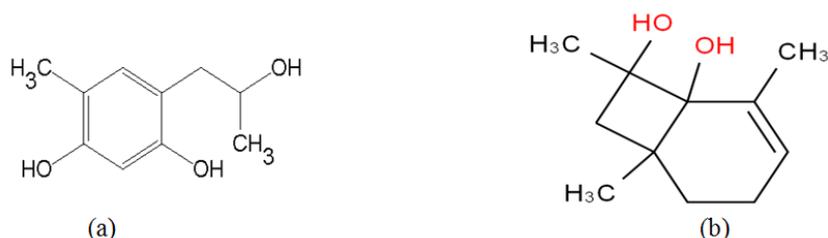


Figure 2. Chemical structures of Phenethyl ethanol, 2,5-dihydroxy-alpha-methyl-,(-)- or 2-(2-hydroxypropyl)-1,4-benzenediol (a) and 2, 6, 8-Trimethylbicyclo[4, 2, 0]oct-2-ene-1,8-diol (b).

3.6. Liver Function Parameters

The biomarkers of liver function in control rats and those exposed to snake venom only or in combination with plant extract are presented in table 5 and figure 3. Treatment with snake venom alone resulted in significant increases in the plasma concentrations of albumin, creatinine, total protein,

urea and uric acid of the treated rats when compared with the control. Conversely, co-administration of plant extract significantly decreased the plasma concentrations of creatinine, total protein and uric acid and maintained the physiological integrity of the liver in venom-treated rats when compared with the group given venom only.

Table 5. Effects of *N. nigricollis* venom, extract and their combination on plasma concentrations of albumin, total protein, creatinine, urea and creatinine in male rats.

Parameters	Control	N. Saline	Ven. Only	Ext. Only	Ven. + Ex.
ALBUMIN	1.81±0.02	1.79±0.08	1.69±0.02 ^a	3.22±0.19 ^a	2.16±0.45
CREATININE	0.36±0.02	0.29±0.12	3.18±0.23 ^a	0.44±0.02 ^a	1.51±0.16 ^b
TOTAL PROTEIN	5.18±0.22	5.22±0.32	3.50±0.47 ^a	4.91±1.19	4.46±0.14 ^b
UREA	11.3±1.48	10.38±0.18	20.58±3.12 ^a	10.49±0.04	13.26±2.97
URIC ACID	3.95±0.93	4.59±0.90	6.67±0.36 ^a	3.60±0.12	3.91±0.37 ^b

N. saline, normal saline, Ven., venom, Ext., extract, Ven. + Ext., venom + extract. The data are presented as mean ±SD for 6 rats per group. a: p<0.05 against control, b: p<0.05 against venom only.

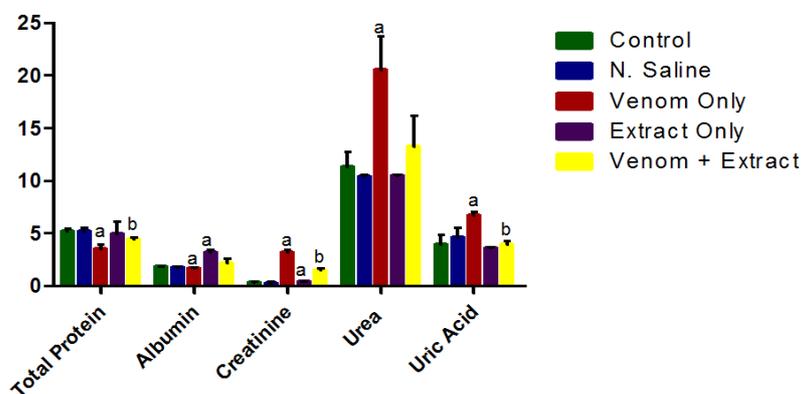


Figure 3. Effects of *N. nigricollis* venom, extract only and their combination on plasma levels of total protein, albumin, creatinine, urea and uric acid in male rats. The data are expressed as mean ±SD for 6 rats per group. a: p<0.05 against control, b: p<0.05 against venom only.

3.7. Enzyme Activity

The effects of venom, plant extract and their combination on the circulatory activities of

ALP, AST and ALP are given in table 6 and figure 4. Exposure to venom only led to significant increases in the

circulatory activities of ALP, AST and ALT in animals when compared with control. However, the circulatory activities of ALP and ALT were significantly reduced in animals co-administered with plant extract when compared with animals exposed to venom only.

Table 6. Effects of *N. nigricollis* venom, extract and their combination on serum activity of ALP, AST and ALT.

Parameters	Control	N. saline	Ven.	Ext.	Ven. + Ext.
ALP	19.44±2.69	21.31±3.10	48.89±0.01 ^a	14.52±4.36	25.73±0.80 ^b
AST	26.95±0.49	26.95±0.99	184.28±5.19 ^a	23.29±4.05	175.88±4.21
ALT	65.8±1.48	71.93±6.19	126.5±0.71 ^a	59.38±0.18 ^a	47.48±4.5 ^b

ALP, alkaline phosphatase, AST, aspartate amino transaminase, ALT, alkaline amino transferase, N. saline, normal saline, Ven., venom, Ext., extract, Ven. + Ext., venom + extract. The data are expressed as mean ±SD for 6 rats per group. a: p<0.05 against control, b: p<0.05 against venom only.

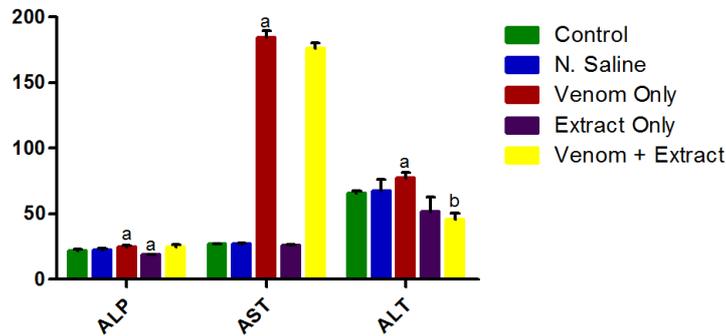


Figure 4. Effects of *Naja nigricollis* venom, extract only and their combination on the serum activities of ALP (alkaline phosphatase), AST (aspartate amino transferase) and ALT (alanine amino transferase) in male rats. The data are expressed as mean ±SD for 6 rats per group. a: p<0.05 against control, b: p<0.05 against venom only.

3.8. Electrolyte Test

The effects of venom, extract and their combination on circulatory sodium, potassium and chloride in animals are given in table 7 and figure 5. There were significant increases in the circulatory concentrations of sodium, potassium and chloride in animals exposed to venom only when compared with control animals. These effects were ameliorated in animals co-administered with plant extract when compared with animals treated with venom only.

Table 7. Effects of *N. nigricollis* venom, extract and their combination on serum levels of sodium, potassium and chloride.

Parameters	Control	N. saline	Ven.	Ext.	Ven. + Ext.
Sodium	147.60±0.31	155.67±4.09 ^a	162.43±1.45 ^a	154.96±5.22	153.91±1.92 ^b
Potassium	5.05±0.38	4.96±1.28	12.47±1.75 ^a	3.87±0.80	3.68±0.44 ^b
Chloride	98.89±4.19	104.44±3.85	134.58±1.60 ^a	95.78±2.14	95.00±3.33 ^b

N. saline = normal saline, Ven. = venom, Ext. = extract, Ven. + Ext. = venom + extract. The data are expressed as mean ±SD for 6 rats per group. A: p<0.05 against control, b: p<0.05 against venom only.

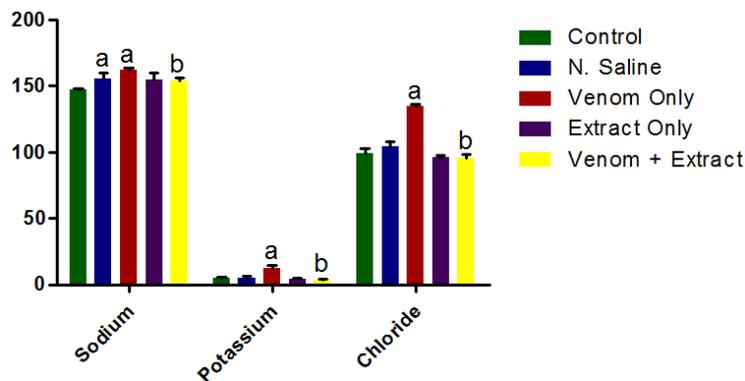


Figure 5. Effects of *N. nigricollis* venom, extract only and their combination on serum levels of sodium, potassium and chloride in male rats. The data are expressed as mean ±SD for 6 rats per group. A: p<0.05 against control, b: p<0.05 against venom only.

4. Discussion

Worldwide, snake envenomation is treated by administration of antivenom. However, antivenom has been in short supply and where they are available they are not affordable. Furthermore, besides the fact that the use of antivenom has a high tendency of causing hypersensitivity reactions in highly sensitive patients, it is also unable to resolve local effects of venom poisoning [16]. Therefore, there is a pressing need for the discovery of novel antivenom from local sources such as medicinal plants. The present study is a work in this direction.

The results of the present study showed changes in protein metabolism and plasma enzyme activities after intraperitoneal administration of *N. nigricollis* venom in rats. The results also showed a disturbance in physiological as well as biochemical activities in experimental animals. Previous studies by researchers showed marked changes in these parameters as well [17].

The present study showed a significant decrease in plasma total protein and albumin levels in rats exposed to the venom when compared with control animals. This reduction could be as a result of disturbance in protein synthesis in hepatocytes due to cellular damage and hemorrhages in vital organs which must have led to protein loss. Such disturbances in protein synthesis have been reported by various researchers with snake venom [4, 17, 18]. Total plasma protein and plasma albumin levels are an indication of liver function. This effect in total protein and albumin levels was significantly modulated in the group of animals coadministered with the plant extract when compared with the group exposed to venom only. This is an indication that the plant extract is a potent antivenom.

Furthermore, the present study also assessed the effects of *N. nigricollis* venom on plasma levels of urea, uric acid and creatinine in rats. The results showed significant increases in plasma levels of creatinine, urea and uric acid in animals exposed to venom only when compared with control rats. These results, except uric acid concentration, are in contrast with results obtained by previous workers [4, 17, 18]. The significant changes in the levels of these substances could be as a result of renal impairment as these parameters are used to assess the functionality of kidney. However, these effects were significantly modulated in group coadministered with the plant extract when compared with rats exposed to venom only. This further potentiates the claim that the extract of *C. multistriata* has antivenom effects.

The circulatory activities of ALP, AST and ALT were significantly increased in rats exposed to the venom of *N. nigricollis* when compared with control animals in the present study. These increases could be as a result of cellular lesions caused by the snake venom on liver, heart, kidney and skeletal muscle. This is an indication that the venom is capable of disrupting the integrity of these tissues. These results are similar to those obtained by other researchers with snake venom experiments [4, 17, 18]. The activities of these

enzymes were significantly modulated in rats coadministered with the methanol leaf extract of *C. multistriata* when compared with rats exposed to venom only. This is an indication that the plant has a protective effect on *N. nigricollis* envenomation.

Data on the effects of snake envenomation on physiologic electrolytes are short in supply. Results of research carried out by Mohammed and coworkers (1964) [19] showed initial decrease in serum sodium and an initial increase in serum potassium levels following *Walterinnesia aegyptia* envenomation in rats. Similar results were obtained with venoms of *W. aegyptia* and *Echis coloratus* in a study by Al-Jammaz (1995) [20]. Omale and coworkers (2013) [4] found an increase in sodium and chloride levels and reduction in potassium, with venom of *N. nigricollis*. The present study found significant increases in the serum concentration of sodium, potassium and chloride in rats exposed to the venom of *N. nigricollis*. These results are in partial agreement with results obtained by Omale and coworkers (2013) [4]. The disturbance in electrolyte metabolism in the rats exposed to the venom could be as a result of acute renal damage as well as glomerular tubular damage. However, these effects in electrolyte metabolism were not observed in rats co-administered with the plant extract.

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is a standard lipophilic radical. The ability of *C. multistriata* to scavenge radicals was estimated from the reduction in absorbance at 517 nm due to scavenging of stable DPPH radicals. The results obtained from the experiment on radical-scavenging activity of the leaf extract is presented in table 3. This positive DPPH test is an indication that the extract is a free radical scavenger. The antivenom activity of the extract can therefore be a result of its ability to scavenge free radicals. The results also showed that the free radical-scavenging activity increases with its concentration. Namely, it is concentration-dependent, which is a favourable property of pharmacological agents.

5. Conclusion

In this study, we were able to identify some bioactive ingredients present in *C. multistriata*. We believe these ingredients and others yet to be identified are responsible for the therapeutic properties of this genus plant. This could be exploited for precise drug targeting against various pathological conditions. The results from the study also showed that *C. multistriata* is a potent antivenom agent against *N. nigricollis* envenomation. We therefore recommend *C. multistriata* as a plant of phyto-pharmaceutical importance in the development of antivenom against snakebite cases arising from *N. nigricollis* envenomation.

References

- [1] Paramonte B. 2007. Snakebites in Nigeria. *Medical Journal of Therapeutics Africa* 1: 222–227.

- [2] Kasturiratne A., Wickremasinghe A. R., de Silva N., *et al.* 2010. The global Snake bite initiative: an antidote for snake bite. *Lancet*, 375: 89-91.
- [3] Nasidi A. 2007. Snakebite as a Serious Public Health Problem for Nigeria and Africa. Presentation to WHO Consultative Meeting on Rabies and Envenomings: by Director, Special Duties, Federal Ministry of Health, Project – Coordinator EchiTAB Study Group, UK/Nigeria on 10th of January, 2007.
- [4] Omale James, Ebiloma Unekwujo Godwin, and Idoko Grace Otini 2013. *Uvaria chamae* (Annonaceae) Plant Extract Neutralizes Some Biological Effects of *Naja nigricollis* Snake Venom in Rats. *British Journal of Pharmacology and Toxicology* 4 (2): 41 – 50.
- [5] Amlabu Emmanuel, Ilani Philip, Ajodo Nicholas, Adewusi Folashade, Yakubu Samirat, Cosmos Yakubu Victor, Ache Eunice, Ezekiel Kayode Atonila and Oshiedu Sarah 2016. GC-MS and NMR analysis of the Bioactive Compounds from the Crude Extracts of *Walthria indica* and the Histopathological Changes induced in Albino Rats Challenged with *Naja nigricollis* Venom. *Journal of Coastal Life Medicine* 4 (5): 395-402.
- [6] Fahimi S., Abdollahi M., Mortazavi S. A., Hajimehdipoor H., Abdolghaffari A. H. and Rezvanfar M. A. 2015. Wound healing activity of a traditionally used poly herbal product in a burn wound model in rats. *Iran Red Crescent Med J*; 17(9): e19960.
- [7] Hemmati M., Zohoori E., Mehrpour O., Karamian M., Asghari S., Zarban A., and Nasouti R. 2015. Anti-atherogenic potential of Jujube, saffron and barberry: anti-diabetic and antioxidant actions. *EXCLI J*; 14: 908-915.
- [8] Atanasov A. G., Waltenberger B., Pferschy-Wenzig E. M., Linder T., Wawrosch C., Uhrin P., Temml V., Wang L., Schwaiger S., Heiss E. H., Rollinger J. M., Schuster D., Breuss J. M., Bochkov V., Mihovilovic M. D., Kopp B., Bauer R., Dirsch V. M., Stuppner H. 2015. Discovery and resupply of pharmacologically active plant-derived natural products: a review. *Biotechnol Adv*; 33 (8): 1582-614.
- [9] Åmand, L. E., Kassman, H., Karlsson, M., Leckner, B., 1997. Measurement of the Concentration of Ammonia and Ethene in the Combustion Chamber of a Circulating Fluidized-Bed Boiler, *Journal of the Institute of Energy*, 70, pp 25-30.
- [10] Omale James, Daikwo Moses Alilu and Musa Achimugu Dickson 2006. Phytochemical Characterization and Biochemical Studies of *Cissus multistriata* Extract Administered to *Rattus norvegicus*. *Animal Research International* 3 (2): 481–484.
- [11] Enemuor, S. C., Omale, J. and Ekpa, M. 2011. Antibacterial Activity: A Comparison of Ripe and Unripe Fruit Extract of *Cissus multistriata* (Vitacea plant). *JBR*; 3 (5): 83–87.
- [12] Still W. C., Khan M. and Mitra A. J. 1978. Rapid Chromatographic Techniques for Preparative Separation with Moderate Resolution. *Org. Chem.* 43 (14): 2923–2935.
- [13] Brett J. W. and Shixin D. (2010). Thin Layer Chromatography Methods for Rapid Identity Testing of *Morinda citrifolia* L. (Noni) Fruit and Leaf. *Adv. J. of Food Sc. and Tech.* 2 (5): 298-302.
- [14] Lisa, B., Francois, H., van der Westhuizen and Du Toit Loots (2008). Phytochemical Contents and Antioxidant Capacities of Two *Aloe great headii* var. *davyana* Extract. *Mol.* 13: 2169–2180.
- [15] Blois M. S. 1958. Antioxidant determinations by the use of a stable free radical, *Nature*, 181: 1199-1200.
- [16] Mahanta, M. and Mukherjee, A. K. 2001: Neutralization of Lethality, Myotoxicity and Toxic Enzyme of *Naja kaouthia* venom by *Mimosa pudica* root extracts. *Journal of Ethnopharmacology* 75: 55–60.
- [17] Malleswari M., Josthna P., and Jacob Doss 2015. Orally Administered Venom of *Naja Naja* Alters Protein Metabolic Profiles in the Liver of Albino Rats. *International Journal of Life Sciences, Biotechnology and Pharma Research* 4 (1): 10–16.
- [18] Goje L. J., Aisami A., Maigari F. U., Ghamba P. E., Shuaibu I. and Goji A. D. T. 2013. The Antisnake Venom Effects of the Aqueous Extracts of *Boswellia delzielli* Stem Bark on the Parameters of Hepatic Functions and Energy Metabolism of *Naja nigricollis* (Spitting Cobra) Envenomed Albino Rats. *Res. J. Chem. Env. Sci.*, 1 (4): 61–68.
- [19] Mohammed, A. H., El-Serougi, M. S. and Karmel, A. M. 1964. Effects of *walterinnesia aegyptia* venom on blood sodium: Potassium and cute cholamines and urine 17-ketosteroids. *Toxicon*, 2: 103-107.
- [20] Al-jammaz, L., 1995. Effects of the venoms of *Walterinnesia aegyptia* and *Echis coloratus* on solute levels in plasma of albino rats. *J. King Saudi Univ., Sci.*, 7 (1): 63-39.