Original Research Article

CHARACTERIZATION AND EVALUATION OF THE EFFECTS OF *INDIGOFERA PULCHRA, ARISTOLOCHIA ALBIDA* AND*ANDROGRAPHIS PANICULATA* LEAVE EXTRACT PHENOLICS AGAINST THE ACTIVITY OF *NAJA NIGRICOLLIS* AND *ECHIS OCELLATUS* SNAKE VENOMS

ABSTRACT

With increased incidence of snake envenomation, high cost of venom antiserum; its adverse side effects and lack of storage facilities for antiserum especially in rural areas, the use of plants as alternatives for treatment of poisonous snakebites is important, especially in these remote areas. This research was aimed at evaluation and characterizationthe effects of *Indigofera pulchra, Aristolochia albida* and*Andrographis paniculata*leave extract phenolics against the activity of *Najanigricollis* and *Echis ocellatus* snake venoms. The plants samples where extracted using chloroform, after which a qualitative and quantitative phytochemical analysis was done, followed by characterization analyses (GC-MS and FTIR). Prep and analytical TLC analyses was carried out on all the extract, where flavonoids and tannins fractions where isolated, using garlic and tannic acids as standard. In-vitro inhibition analyses of the partially purified phenolicswas done to ascertain the effects to the isolated phenoilic fractions against the two selected crude snake venoms. The plant extracts characterization done reveals that all the three extracts contain phenolics and specifically important compounds like, Benzaldehyde-2-hydro-4-methoxy, rutin and gallocatechin, all which has been reported to have anti-snake venom capability. The inhibition studies carried out reveals that the flavonoid fractions of the extracts has a higher inhibitory effect against the snake venoms than the tannin fractions of all the extracts. Characterization and evaluation studies, done in this research has reveals that these plants phenolic fractions has effects on the two snake venoms and can help in the management and treatment of snake bite.

INTRODUCTION

Plants have been for long seen and exploited as potential source of medical agents and can be traditionally used to treat many disease and infections especially infectious disease including diarrhea, fever, cold and numerous infections (Audu*et al.*, 2007). Plants can however be also use in the treatment and management of zoonotic hazards such as bites from snakes, bees, scorpions

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and other zoonotic animals. Many compounds used in traditional and modern medicine, has one or more plant source material. These compounds can also be used as a pioneer, in the synthesis of semi-synthetic drugs, serving as source of food and medicine for human and animals (Hassan *et al.*, 2021).

These plant compounds, with medicinal capabilities are known and referred to as Phytochemicals. These are also referred to as phyto-metabolites. These are however chemical compounds produce by plants, to aids them depend or fight against competitors, predators, or pathogens (Das, 2010). The name originates from a Greek word 'phyton', meaning "plant". Some phytochemicals have been used as poisons and some as traditional or local medicines. These compounds are basically classified into two; primary metabolites and secondary metabolites (Obadoni and Ochuko 2001). The name phytochemicals is used to describe plant compound that are under research with unknown effects on health and are not scientifically defined as essential nutrients. They are commonly found in fruits, vegetables, nuts, legumes, herbs, grasses and trees (Nikhal*et al.*, 2010). Phytochemicals are usually confused with phytonutrients, but phytochemicals include plants compound that are useful and those that harmful as well, while phytonutrients specifically refers to plant compound that have positive effect, in other word all phytonutrients are phytochemicals, but it is not all phytochemicals that are phytonutrients (Paulchamy*et al.*, 2010). Therefore the difference between phytochemicals and phytonutrients is quite essential, as not all phytochemicals are beneficial (Mulu *et al.*, 2008). These chemicals are normally accumulated and concentrated in different part of the plants, such as in the fruits, flower, leaves, stem or roots. Many phytochemicals particularly the pigment molecules are often concentrated in the outer layers of the various plant tissues level vary from

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plant to plant depending upon the variety, processing, cooking and grooving condition (Solomon *et al.*, 2004).

Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acid and chlorophylls (Vidyadhar*et al.*, 2010). While the secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics and glucosides (Handa*et al.*, 2008). Literature survey indicate that phenolics are the most numerous and structurally diverse plant phytoconstituents (Obasi *et al.*, 2010).Several health benefits have been recognized for the intake of flavonoids and tannins this includes, some epidemiological associations with the decreased frequency of chronic diseases and zoonotic anti venom activity, with an emphasis on snake envenomation (Serrano *et al.*, 2009). Several medicinal plants have and are being used in the treatment and management of snake envenomation locally. These include; *Guinea senegalensis*, *Acalypha indica*, *Tamarindus indica* and some few others, all which are known to aid in neutralization of varieties of snake venom toxicity (Vineetha*et al.*, 2017).With increased incidence of snake envenomation, high cost of venom antiserum; its adverse side effects and lack of storage facilities for antiserum especially in the usually remote snake endemic areas of Nigeria. The use of plants as alternatives for treatment of poisonous snakebites is important in remote areas where there is no accessibility to hospitals and storage facilities for snake venom antiserum (Hassan *et al.*, 2020). Efforts are continuously being made to develop alternative treatment strategy from medicinal plants (Santosh. 2004). This research was focused on evaluating the effect of *Indigofera pulchra*, *Aristolochia albida* and*Andrographis paniculata*leaveextractsfractions against the activity of *Najanigricollis* and *Echis Ocellatus* snake venoms.

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MATERIALS AND METHOD

Collection and Identification of Plant Materials

I. pulchra, A. albida and*A. paniculata*leaves were collected from Malumfashi LGA, Katsina state, Nigeria. Its botanical identity was further confirmed and authenticated at the herbarium section of the Department of Biological sciences, Nigerian Defence Academy, Kaduna.

Snake Venom Sample Collection

Lyophilizedvenom of *E. ocellatus* and N. nigricollis(400mg each)was purchased from the snake lab of Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Kaduna Nigeria and was aseptically transported and stored at -4^{0}C until used.

Preparation and Treatment of Plant Samples

The leaves were surfaced sterilized, air dried under shade and ground to powder using mortar and pestle and stored in an air tight container as described by Lakache, (2016).

Plant Material Extraction Protocol (Maceration)

This was carried out according to the method ofKumar, (2009), using chloroform as the extraction solvent. The fine powder of leaves (290g each) were weighed and macerated in an amber maceration bottle (with regular sharking) for 7days. After which the mixture were filtered, using fine cotton sieving material and a KNF Neuberger vacuum suction pump was used to enhance filtration to separate the liquid sample from the solid residue. The liquid mixture were finally evaporated (using water bath at 40^{o}C), weight and stored in sterile air tight containers.

Phytochemical Screening

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Quantitative and qualitative phytochemical analyses was carried out using standard procedures as described by Velavan, (2015).

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Thin Layer Chromatography (TLC)

Analytical Thin-layer Chromatography

TLC was done according to the method of Lihua*et al.*, (2009). A 10×1.5 cm TLC plates were coated and activated by heating at 110^{o}C for 60 min and allowed to cool to room temperature. Pencil lines were drawn 1.5 cm from one edge of the plates. Extract samples were then spotted using thin capillary pipettes onto the pencil line. The plates were placed in a development chamber with a trial solvent. The solvent front was allowed to travel until about 1 cm from the top end. The TLC plates were removed and solvent front marked using a soft pencil. These wereair dried and then sprayed with a fine spray of 1% ethanolic aluminum chloride solution, left to dry and then visualized under UV light at 365 nm. The chromatograms were marked and retention factors calculated and recorded.

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Preparative Thin-layer Chromatography

Pre-coated thick silica gel on glass TLC plates measuring 20×20cm were used. The chloroform/hexane (8:2, v/v) mobile phase solvent system was used and each of the Chloroform extracts from the samples were deposited as a concentrated band 1.5cm from the edge of its respective TLC plate and allowed to dry. The plates, with dried samples, were gently lowered into the development tank, closed and left to develop. The plates were then removed from the development chamber when the solvent front had traveled three quarters of the plate's length.

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The position of the solvent front was immediately marked with a soft pencil. The retention factor (R_f) values of the different bands were then calculated using the equation:

R_f = Ratio of the distance the spot move above the origin to the distance the solvent moved above the origin (Hassan *et al.*, 2020).

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Using the method reported by Mittal, (2013), the bands that tested positive against flavonoids and tannins standard were scratched off, re-tested and mixed with 5 ml of absolute chloroform, allowed to stand for 10 min and then filtered with Whatman No.1 filter paper and collected in glass vials.

Extract Evaluation Analysis

GC-MS and FTIRanalysis were carried out using standard procedures as described bySoladoye, (2012)and Saxena, (2013) respectively.

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Spectrometric Maximum Wave Spectral Scanning

Spectral spectrometric scanning analysis was done on flavonoids and tannins standard (garlic and tannic acids) at 260nm against the partially purified phenolics fractions to ascertain which of the fractions has similar compounds with the standard (Hassan *et al.*, 2020).

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Venom Protein Inhibition Studies

This is carried out using standard procedures as described by Nwune, (2016), were the total protein concentration of the crude venom was tested prior and after addition of the partially purified phenolics.

Statistical Analysis

Some of the data obtained were presented as mean ± standard deviation of three determinations. The analysis of variance was used to compare the paired means; the $P < 0.05$ was considered statistically significant.

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RESULTS

Result of plants sample extraction of all the three plants, shows the physical properties and percentage yieldof the extracts as shown in table 1. While the qualitative and quantitative phyto-metabolic analysis done reveals that, *I. pulchra* is devoid of phytosteroids, coumarin and contain Saponins (9.484 ± 0.220) as the highest containing phytochemical. While that of *A. albida* shows that the extract is devoid of metabolites like Cardiac glycoside, quinines and has phenols (9.320 ± 1.260) as the highest containing phytochemical. That of *A. paniculata* however shows that the extract is devoid of Coumarins, vitamin A and has alkaloids (15.271±0.1072) as the highest containing phytochemical. GC-MS and FTIR analyses where also done on all the extracts, which reveals the various compounds and functional groups of the individual extracts as shown in table 5 – 10. Prep and analytical TLC analyses was carried out on all the extract, where flavonoids and tannins fractions where isolated, using garlic and tannic acids as standard. The standards where however also used in carrying out a re-confirmatory Spectrometric Maximum Wave Spectral Scanning analyses to further confirm the fractions as shown in table 12. Lastly an In-vitro inhibition analyses of partially purified phenolics was done against the two selected crude snake venoms.

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Table 1: Percentage yield and physical properties of*I. pulchra* and *A. albida* chloroform extracts

Plant Material	Initial Weight of Plant Material (g)	Total yield (g)	Yield (%)	Colour	Texture
I. pulchra	290	19.25	6.64	Dark greenish	Gummy
A. albida	290	51.99	17.9	Light green	Crystalline
A. paniculata	290	23.50	8.1	Light green	Crystalline

Table 2: Qualitative and Quantitative Phytochemical Content of *I. pulchra* Chloroform Leave Extract

S/N	Phytochemical	Qualitative	Quantitative (mg/g dry wt)
1	Flavonoid	+	8.130 ± 2.452
2	Alkaloid	+	5.553 ± 0.957
3	Saponins	+	9.484 ± 0.220
4	Phytosterols	-	
5	Phenols	+	8.947 ± 1.020
6	Terpenoids	+	1.267 ± 1.521
8	Triterpenoids	+	1.503 ± 0.021
9	Tannins	+	9.310 ± 3.836
10	Cardiac glycoside	+	1.540 ± 0.151
11	Anthraquinones	+	0.095 ± 0.102
12	Anthocyanins	_	
13	Phlobatannins	+	
14	Flavonols/flavones	_	
15	Coumarins	_	
16	Quinones	_	
17	Resins	+	
18	Amino acids	+	
19	Chalcones	+	
20	Vitamin A	_	

21	Vitamin D	+
22	Acidic compound	+

Key:
+ = Presence - = Absence
Results are presented as mean ± standard deviation

Table 3: Qualitative and Quantitative Phytochemical Content of *A. albida* Chloroform Leave Extract

S/N	Phytochemicals	Qualitative	Quantitative (mg/g dry wt)
1	Alkaloid	+	0.931 ± 1.707
2	Flavonoid	+	2.955 ± 0.021
3	Saponins	+	4.391 ± 1.072
4	Phytosterols	+	
5	Phenols	+	9.320 ± 1.260
6	Terpenoids	+	0.090 ± 0.002
7	Tannins	+	2.732 ± 0.151
8	Triterpenoids	+	1.434 ± 0.343
9	Cardiac glycoside	-	0.941 ± 0.011
10	Anthraquinones	+	1.712 ± 0.031
11	Anthocyanins	+	
12	Phlobatannins	-	
13	Flavanols and flavones	+	
14	Coumarins	+	
15	Quinines	-	
16	Chalcones	-	
17	Steroids	+	
18	Vitamin A	_	
19	Vitamin D	-	
20	Acidic compound	+	
21	Resins	+	
22	Amino acids	-	

Key:
+ = Presence - = Absence
Results are presented as mean ± standard deviation

Table 4: Qualitative and Quantitative Phytochemical screening of Chloroform Leaf Extract of *A. paniculata*

S/N	Phytochemicals	Qualitative	Quantitative (mg/g dry wt)
1	Alkaloid	+	15.271±0.1072
2	Flavonoid	+	0.823±0.1701
3	Saponins	+	0.215±0.0001
4	Phytosterols	+	
5	Phenols	+	11.143±0.4345

6	Terpenoids	+	
7	Tannins	+	1.632±1.2736
8	Triterpenoids	+	
9	Cardiac glycoside	-	
10	Anthraquinones	+	0.009±0.0002
11	Anthocyanins	+	
12	Phlobatannins	-	
13	Flavanols and flavones	+	1.574±0.0151
14	Coumarins	-	
15	Quinines	-	
16	Chalcones	-	
17	Steroids	-	
18	Vitamin A	_	
19	Vitamin D	-	
20	Acidic compound	+	
21	Resins	-	
22	Amino acids	-	

Results are in mean ± standard deviation.

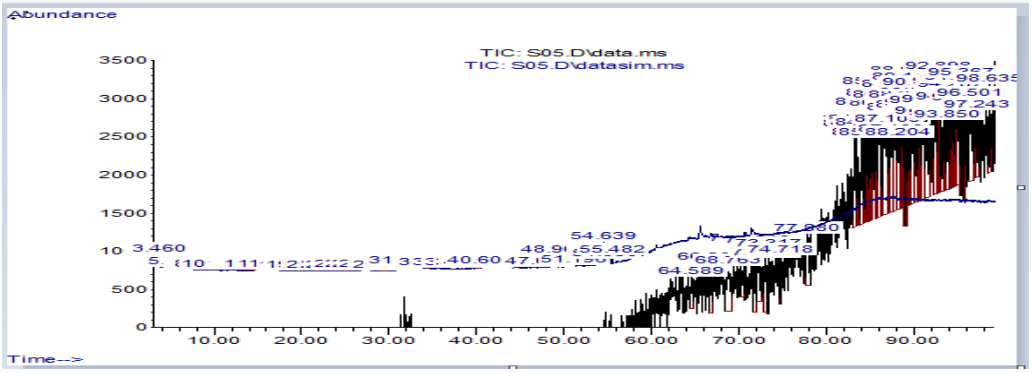


Figure 1a: GC-MS Analysis Micrograph of *A. albida* Chloroform Leave Extract

Table 5: Probable peaks obtained from the GC-MS analysis of *A. albida* Chloroform Leave Extract

PK	RT	AREA	LIBRARY/ID	QUALITY
1	64.589	1.05	Urea	2
2	66.801	1.15	Hydrazine-1,2-dimethyl	2
3	68.763	2.38	Thiirine	2
4	70.319	1.15	Carbonyl sulfide	2
5	71.810	1.35	Hydrazine-1.1-dimethyl	2
6	72.249	1.67	Carbonyl sulfide	2
7	72.974	1.47	Acetic acid	2
8	73.317	1.22	Hydrazine-1,2-dimethyl	2
9	74.718	1.11	Urea	2
10	77.880	2.11	Propanamide	4
11	83.181	1.87	Isobutylamine	3
12	83.483	1.43	Hexanoicn acid-6-hydroxy	4
13	84.008	2.11	Isobutylamine	4
14	84.273	1.56	Carbamodithioc acid, formyl, methyl ester	5
15	84.501	1.56	Ethane, methoxy-	4
16	84.745	1.19	Ethyl ether	4
17	84.894	1.14	Acetic acid,(aminooxy)	7
18	85.204	1.20	Guanidine, methyl-	3
19	85.685	2.24	7- octenoic acid	4
20	86.036	4.28	5- chlorovaleric acid	4
21	86.392	1.80	Hexanoic acid-6-hydroxy-	4
22	86.726	3.74	Propanamide	3
23	87.109	1.72	Benzaldehyde-2-hydro-4-methoxy	3

24	87.400	2.24	Propanamide	4
25	87.816	3.93	Acetic acid,(aminooxy)-	4
26	88.446	1.65	Propanamide	3
27	88.446	1.77	Thiirine	4
28	88.645	1.65	2-(p-tolyl)ethylamine	3
29	88.916	1.64	Propanamide	5
30	89.110	3.40	Guanidine, methyl-	3
31	89.491	1.52	Guanidine, methyl	3
32	89.783	2.98	Isobutylamine	4
33	90.168	2.53	Guanidine, methyl-	3
34	90.502	1.52	2-(p-tolyl) ethylamine	3
35	91.020	4.00	Propanamide	3
36	91.589	3.25	Acetic acid, (aminooxy)-	4
37	92.444	3.31	Guanidine, methyl	3
38	92.898	2.42	2-(p-tolyl) ethylamine	3
39	93.217	1.44	7-octenoic acid	3
40	93.542	2.06	Isobutylamine	4
41	93.850	1.46	Guanidine, methyl	3
42	94.076	1.22	Acetic acid, (aminooxy)-	4
43	94.287	1.83	Isobutylamine	3
44	94.677	1.71	Isobutylamine	4
45	94.977	2.96	N-Acetylethylenediamine	4
46	95.367	1.76	2-(p-tolyl) ethylamine	3
47	95.784	1.19	2-(p-tolyl) ethylamine	7
48	96.501	3.13	Propanamide	3
49	97.243	1.29	Guanidine methyl	3
50	98.635	1.62	Inositol-1-deoxy-	4

PK = Peak, RT = Retention time

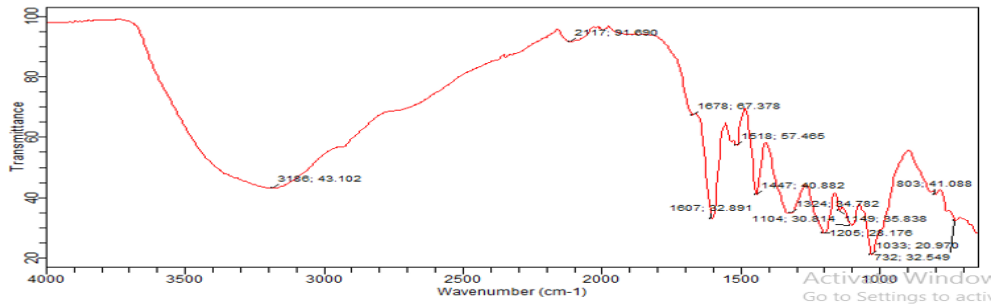


Figure 1b: FTIR Micrograph of *A. albida* Chloroform Extract

Table 6: Probable Functional Groups Obtained from the FTIR Analysis of *A. albida* Chloroform Leave Extract

S/N	Absorption Range (Cm^{-1})	Frequency (Cm^{-1})	Bond (types of vibration)	Functional Group.
1	3300-3200	3188	$\equiv C-H$ Stretch	Alkynes
2	2200-2100	2117	$C \equiv C$ stretch	Alkynes
3	1710-1665	1678	$C=O$ stretch	Unsaturated aldehydes,ketones.
4	1550-1450	1518	N-H bend	Amines-secondary
5	1640-1550	1607	N-H bend	Amides
6	1500-1440	1447	H-C-H bend	Alkanes
7	1360-1290	1324	N-O symmetrical stretch	Nitro compounds
8	1250-1020	1104	C-N stretch	Aliphatic amines
9	1250-1020	1205	C-N stretch	Aliphatic amines
10	1250-1020	1033	C-N stretch	Aliphatic amines
11	850-550	732	C-CL stretch	Alkyl halides
12	900-675	803	C-H "oop"	Aromatic compounds
13	1300-1000	1149	C-O stretch	Ethers

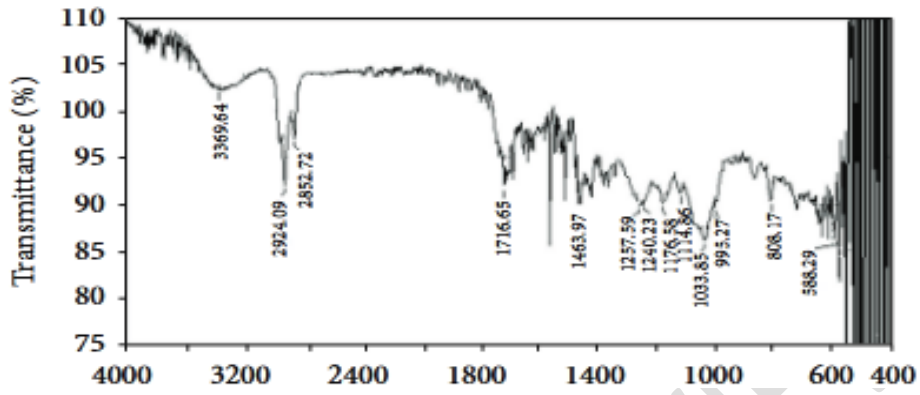


Figure 2a: FTIR Micrograph of *I. pulchra* Chloroform Extract

Table 7: Probable Functional Groups Obtained from the FTIR Analysis of *I. pulchra*Chloroform Leave Extract

S/N	Absorption Range (Cm^{-1})	Frequency (Cm^{-1})	Bond (types of vibration)	Functional Group.
1	3500-3300	3369.64	OH group (alcohol)	OH stretching, H-bonded
2	2950-2600	2924.09	CH Alkanes	C-H stretching alkanes
3	2860–2660	2861.80	CH Alkanes	C-H stretching alkanes
4	2860–2660	2852.72	Ester group	C=O ester stretching
5	1745-1550	1716.65	Aromatic C=C group	C=C stretching
6	1500-1470	1463.97	Methylene group	C-H bending
7	1380-1290	1257.59	OH group (alcohol)	OH stretching
8	1250-1020	1240.23	C-O Carboxylic Acid	C-O ester stretching
9	1300-1000	1176.58	C-O stretch	Ethers
11	1000-850	1033.85	C-CL stretch	Alkyl halides
12	1000-850	995.27	O–H bend	Carboxylic Acids

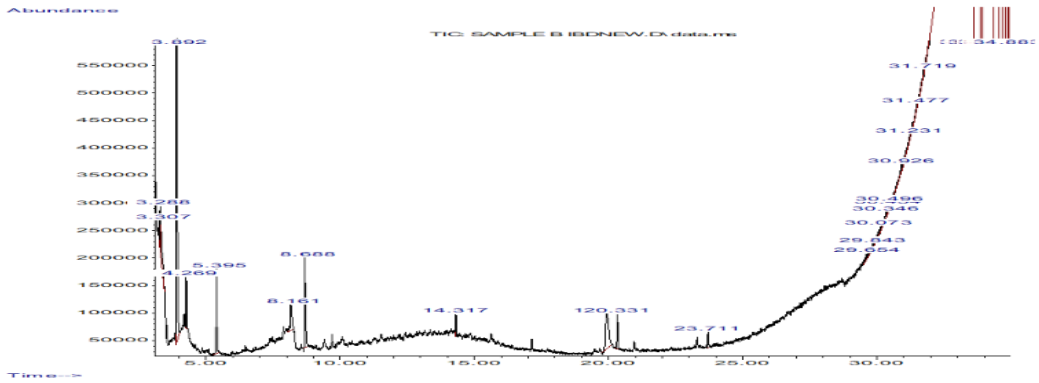


Figure 2b: GC-MS Micrograph of *I. pulchra* Chloroform Leave Extract

Table 8: Probable peaks obtained from the GC-MS analysis of *I. pulchra* Chloroform Leave Extract

PK	RT	AREA	LIBRARY/ID	QUALITY
1	279	4.91	(2E,4E)-N-Isobutyltetradeca-2,4-dienamide (C18H33NO)	5
2	116	2.38	Pentanoic acid, 3-methyl-	2
3	151	1.15	Rutin	3
4	283	3.35	alpha.-Benzamido-2-hydroxycinnamic acid(C16H13NO4)	7
5	89	1.67	N,N-Dimethylaminoethanol (C4H11NO)	2
6	172	2.47	1,1,2-Trimethyl-3,8,9-trioxa-bicyclo [4.2.1]nonane (C9H16O3)	2
7	298	1.22	Methyl stearate (C19H38O2)	2
9	193	2.11	1-(4-Methoxy-3-methylphenyl)-2-methylpropan-2-amine (C12H19NO)	4
10	126	4.16	Maltol (C6H6O3)	
11	180	2.38	Theobromine (C7H8N4O2)	2

12	214	1.15	Dodecanoic acid, methyl ester (C13H26O2)	2
13	270	1.35	Hexadecanoic acid, methyl ester (C17H34O2)	4

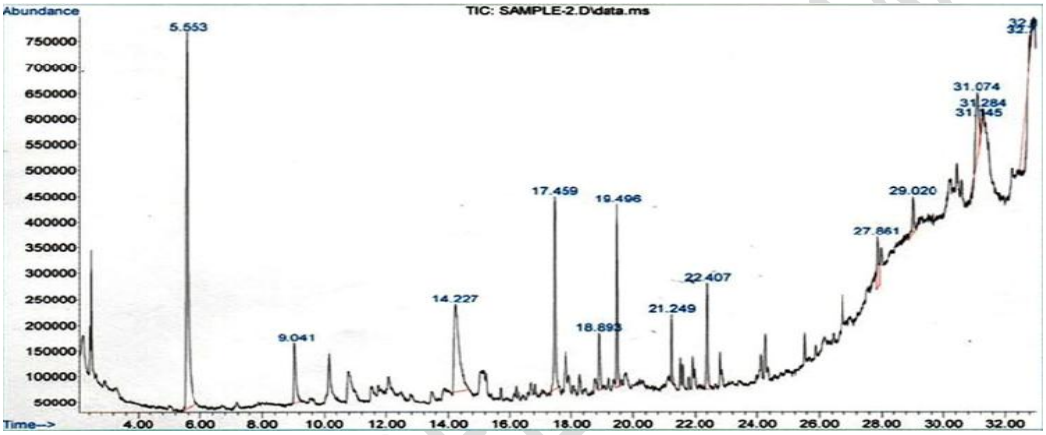


Figure 3a. GC-MS Micrograph of *A. paniculata*Chloroform Leave Extract

Table 9: Probable peaks obtained from the GC-MS analysis of *A. paniculata* Chloroform Leave Extract

PK	RT	AREA	LIBRARY/ID	QUALITY
1	5.568	3.43	Furfural ($C_2H_4O_2$)	3
2	22.407	2.52	Hexa decanoic acid – methyl ester ($C_{17}H_34O_2$)	5
3	9.041	1.75	Carboxaldehyde, 5-methyl ($C_6H_6O_6$)	2
4	21.249	2.15	Carbamodithioc acid, formyl, methyl ester	3

5	14.227	2.51	2-FuranCarboxaldehyde-5-(hydroxyl methyl) ($C_6H_6O_3$)	2
6	29.020	3.97	Acetic acid,(aminooxy)	4
7	17.459	1.79	Benzaldehyde-2-nitroso	2
8	18.893	1.15	Gallocatechin	2
9	19.496	3.43	Benzyle chloride ($C_6H_5CH_2Cl$)	2
10	27.861	2.43	2-(p-tolyl) ethylamine	3
11	31.074	1.28	Guanidine methyl	5
12	31.284	1.44	7-octenoic acid	4

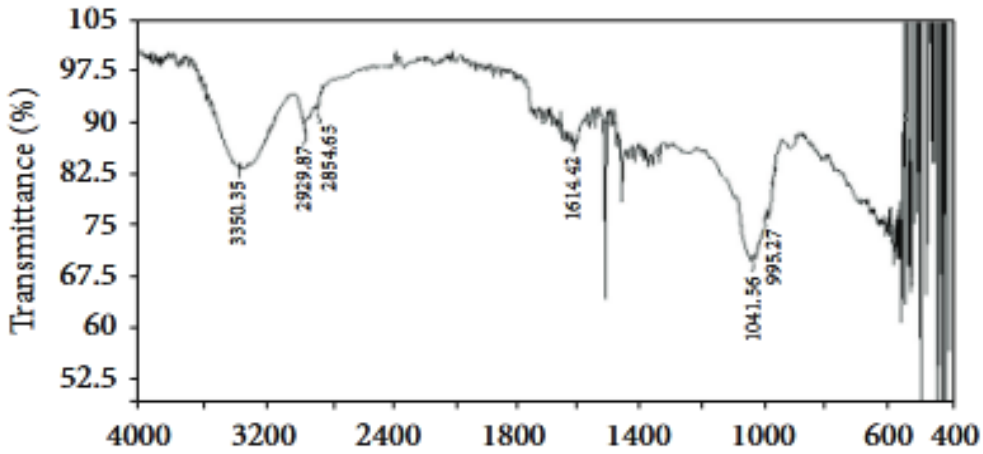


Figure 3b: FTIR Micrograph of *A. paniculata* Chloroform Extract

Table 10: Probable Functional Groups Obtained from the FTIR Analysis of *A. paniculata* Chloroform Leave Extract

S/N	Absorption Range ($Cm^{-}1$)	Frequency ($Cm^{-}1$)	Bond (types of vibration)	Functional Group.
1	3350-3200	3350.35	N–H stretch 1∘, 2∘	amines, amides
2	3000-2700	2929.87	C–H stretch	Alkanes
3	3000-2700	2854.87	C–H stretch	Alkanes
4	1640-1550	1614.47	C=O stretch	Carboxylic acid
5	1250-1020	1041.57	C-N stretch	Aliphatic amines
6	1250-9050	995.27	=C–H bend	Alkenes

Table 11a: THIN LAYER CHROMATOGRAPHY RESULT (TLC of Standards)

S/N	Standard	R_f Value
1	Garlic Acid	0.918 0.82
2	Tannic Acid	0.75 0.69

Table 11b: *A. albida* Leaf Extract TLC Analysis: Solvent font: 13.5cm

S/N	FRACTIONS	FRACTION DISTANCE (cm)	Rf VALUE
1	Fraction 1	12.4	0.92
2	Fraction 2	11.8	0.87
3	Fraction 3	10.4	0.77
4	Fraction 4	9.4	0.69
5	Fraction 5	7.9	0.58
6	Fraction 6	6.3	0.46
7	Fraction 7	3.2	0.24
8	Fraction 8	2.0	0.15
9	Fraction 9	1.8	0.13
10	Fraction 10	1.4	0.10
11	Fraction 11	1.2	0.09

Table 11c: *I. pulchra* Leaf Extract TLC Analysis: Solvent font: 15.3cm

S/N	FRACTIONS	FRACTION DISTANCE (cm)	Rf VALUE
1	Fraction 1	13.2	0.86
2	Fraction 2	11	0.72
3	Fraction 3	7.2	0.47

4	Fraction 4	3.1	0.20
5	Fraction 5	2.3	0.15
6	Fraction 6	1.6	0.10
7	Fraction 7	1.4	0.09
8	Fraction 8	0.9	0.06

Table 11d. *A. paniculata* Leaf Extract TLC Analysis: Solvent font: 13.1cm

S/N	FRACTIONS	FRACTION DISTANCE (cm)	Rf VALUE
1	Fraction 1	11.4	0.87
2	Fraction 2	10.2	0.78
3	Fraction 3	8.2	0.62
4	Fraction 4	7.1	0.54
5	Fraction 5	5.3	0.4
6	Fraction 6	2.6	0.2
7	Fraction 7	1.4	0.1
8	Fraction 8	1.1	0.08

Table 12: Spectrometric Maximum Wave Spectral Scanning of Standard/Plant Extracts Fractions from TLC

	*	Fractions	Maximum Wave Spectra(nm)
Standards	Garlic Acid		292.5
	Tannic Acid		310
	A. albida	1	**290[1]***
		2	**305[2]***
		3	284.5

	4	274.5
	5	298
	1	299
I. pulchra	2	**293**[1*]
	3	**309**[2*]
	4	298
A. paniculata	1	**291**[1*]
	2	**305**[2*]
	3	300

Key:
1*: Positive Flavonoid Fraction
2*: Positive Tannin Fraction

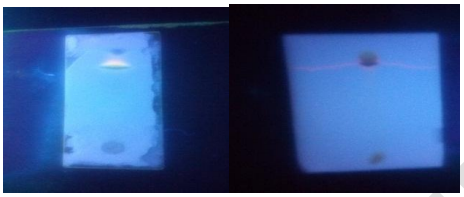
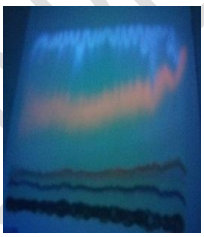


Figure 4a: TLC Plates for Standard (Garlic Acid and Tannic Acid)

A.  B.  C. 

Figures 4b: TLC plates of ***A. albida,*** *A. paniculata*and *I. pulchra* Leaf Extracts

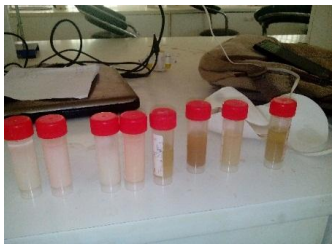
1.  2.  3. 

Figure 4c: Extracts fractions of ***A. albida,*** *A. paniculata*and *I. pulchra*

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Table 13: In-vitro inhibition analyses of the partially purified phenolics against the two selected crude snake venoms

Snake venom	Crude Venom Total protein (mg/ml)	Plant	Plant Fraction	Total Protein (mg/ml)
E. ocellatus	0.643265 ± 0.015776	*A. albida*	PPF	0.321071
			PPT	0.521202
		I. pulchra	PPF	0.298013
			PPT	0.459333
		A. paniculata	Crude Extract	0.412966
N. nigricollis	0.363426 ± 0.012281	*A. albida*	PPF	0.234289
			PPT	0.310951
		I. pulchra	PPF	0.194535
			PPT	0.222817
		A. paniculata	Crude Extract	0.262879

Key:
PPF: Partially Purified Flavonoids
PPT: Partially Purified Tannins

DISCUSSION

Snake envenomation has for been an issue of medical and medical importance. And it happens that the only medical treatment for snake bite is by parental administration of biosynthesized antiserum, which is associated with administration, dosage, side effect and storage problems (which clearly requires further medical research). Since development of snake venom antiserum

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and its standardization are found to be expensive, difficult and require ideal storage conditions (Theakson*et al.,* 2003); which are not available in the usually remote snake endemic areas of Nigeria.

With increased incidence of snake envenomation, high cost of venom antiserum; its adverse side effects and lack of storage facilities for antiserum especially in rural areas, the use of plants as alternatives for treatment of poisonous snakebites is important, especially in these remote areas where there is no much accessibility to hospitals and storage facilities for snake venom antiserum. Some ethno-plants materials are normally used traditionally, in the management and treatment of snake envenomation. However some researchers have reported that plants extracts phenolics due have some anti-snake venom capabilities (Gomes *et al.*,2010).

In this study the efficiency of the phenolic extracts of *A. albida*, *A. paniculata*and *I. pulchra*where tested against *E. ocellatus*and *N. nigricollis* in-vitro.The phytochemical analysis of the plant extracts done in this study revealed the presence of tannins, saponin, alkaloids, flavonoids, amino, phenols, triterpenoids and terpenoids in all the three plant extracts tested.Which are among the phytometabolites reported to have anti-snake venom potency (Grish*et al.,* 2004). The GC-MS and FTIR analyses shows that the extracts have compounds and functional groups like, Benzaldehyde-2-hydro-4-methoxy (a Phenolic) in the *A. albida* extract, rutin in *I. pulchra*and gallocatechin in *A. paniculata*extract, all which has been reported to have some anti-snake venom potentials (Isabel *et al.,* 2019).The standards where however also used in carrying out a re-confirmatory Spectrometric Maximum Wave Spectral Scanning analyses to further confirm the fractions as shown in table 12 against garlic and tannic acids as flavonoid and tannin standards. In-vitro inhibition analyses of the partially purified phenolics done against the

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two selected crude snake venoms, reveals that the extracts has some positive effects on the venom total protein. The flavonoids fractions of the extract however shows a more better activity against the venoms than the tannins fractions of all the extracts, all as shown in table 13.*I. pulchra*flavonoid fraction however has the highest activity against both the *E. ocellatus* and *N. nigricollis*snake venoms.

This study was compared to research done by *Lans et al.* (2001), where he stated that 'phytochemicals due inhibitsvenom phospholipase A_2 activities of both viper and cobra venom. Phenolics, especially polyphenols like some tannin, bind proteins acting upon the component of venom directly and disabling them to act upon the receptors', and they could also act by competitive blocking of the receptorsEvans *et al.,* (2002).

Gomes *et al* (2010) reported that the herbal constituents are active against snake envenomation including among others; alkaloids, steroids, tannins, flavonoids and terpenoids. Okonogi*et al* (1979) suggested that tannins in addition to other plant constituents which are known to un-specifically inactivate proteins to be the likely mechanism involve in detoxifying the snake venom. Evans *et al* (2002) reported that tannins precipitate proteins and form dark-coloured complexes with metals such as iron.Similar studies was conducted by Ushanandini*et al.* (2006), which indicated that*Tamarind* seed extract inhibited the activity ofsnake venom proteins like; PLA_2, protease, hyaluronidase, l-amino acid oxidase and 5'-nucleotidasein a dose-dependent manner.

CONCLUSION

A. albida, *A. paniculata*and *I. pulchra*phenolic extracts fractions could provide an alternative natural remedy for the management and treatment of snakebite.

REFERENCES

Comment [DDM62]: format should be uniform

Audu SA, Mohammed I. and Kaita HA. (2007), Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). *Life Science Journal*; 4(4): 75-79

Das K, Tiwari R. and Shrivastava DK. (2010), Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*; 4(2): 104-111.

Evans WC and Sannders WB. (2002), Plants in African traditional medicine: *An overview in trease and evans pharmacognosy*. 15th ed. London: Saunders. Pp 488–495.

Girish K.S, Mohanakumari H.P, Nagaraju S, Vishwanath BS, Kemparaju K. (2004) Hyaluronidase and protease activities from Indian snake venoms: neutralization by Mimosa pudica root extract. *Fitoterapia*; **75**: 378–380.

Gomes A, Das R, Sarkhel S, Mishara R, Mukherjee S and Bhattacharya S (2010), Herbs and herbal constituents active against snake bite. *Indian Journal of Experimental Biology*; 48:865–878.

Handa SS, Khanuja SPS, Longo G. and Rakesh D. (2008). Extraction Technologies for Medicinal and Aromatic Plants. *International centre for science and high technology, 99 Trieste* Italy; 34012(6): 21-25

Hassan A. U., A. H. Madu, U. O. Ozojiofor, A. H. Galadanci, I. B. Mato and R. Jafaru (2021), Antimicrobial Activities of *Cymbopogon citratus* and *Ximenia Americana* Leaf Extracts Against Some Selected Bacterial and Yeast Clinical Isolates. *Asian Journal of Biochemistry, Genetics and Molecular Biology*; 9(1): 1-10, DOI: 10.9734/AJBGMB/2021/v9i130204

Hassan A. U., M. S. Makeri, U. O. Ozojiofor and A. J. Alhassan (2020), Effects of *Ficus sycomorus* Phenolic Extracts on the Activity of Hyaluronidase and Phospholipase A_2 Enzymes of Echis ocellatus Venom. *Asian Journal of Biotechnology and Genetic Engineering*; 3(4): 24-33.

Isabel G. B, Vedanjali G., Andrea S. and Francisco L. (2019) Perspective on the Therapeutics of anti-snake venom. *Molecules*, 24, 3276

Kumar, A., Ilavarasn, R., Jayachandran, T., Decaraman, M., Aravindhan, P. and Padmanaban, N. (2009), Phytochemical investigation on tropical plant. *Pakistan Journal of Nutrition*; 8(1): 83-85.

Lakache Z., Tigrine-Kordjani N., Tigrine C. Aliboudhar H. and Kameli A. (2016), Phytochemical screening and antioxidant properties of methanolic extract and different fractions of *Crataegus azarolus leaves* and flowers from Algeria. *International Food Research Journal*; 23(4): 1576-1583

Lans C. and Harper T (2001), Georges K, Bridgewater E. Medicinal and ethnovetrinary remedies of hunters in trinidad BMC compliment. Alternate Medicine; 1:1–10.

Lihua G, Tao W. and Zhengtao W. (2009), TLC bioautography-guided isolation of antioxidants from fruit of Perilla frutescens var. acuta. Food Science Technology; 42: 6-131.

Mittal M, Berehan T. and Alemu Y (2003), The effect of grass hay with different level of Brewer's Dried Grain on feed intake, digestibility and body weight gain intact Wogera lambs. *East African Journal of Science* 2: 105-110

Mulu M, Berehan T. and Alemu Y (2008), The effect of grass hay with different level of Brewer's Dried Grain on feed intake, digestibility and body weight gain intact Wogera lambs. *East African Journal of Science* 2: 105-110.

Nikhal SB, Dambe PA, Ghongade D. and Goupale DC. (2010), Hydroalcoholic extraction of *Mangifera indica* (leaves) by Soxhletion. *International Journal of Pharmaceutical Sciences*; 2 (1): 30-32.

Nwune Hope Chinyere, Mohammed Adamu Milala and Hassan Zannah (2016), Effects of Aqueous Root Extract of Annona Senegalensis on Bitisarietans Venom Protease and Phospholipase A_2 Activities. *Journal Pharmaceutical Biomedical Science***,** 06(08): 469–473.

Obadoni BO. and Ochuko PO (2001), Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science*; 8(1): 203-208.

Obasi NL, Egbuonu AC, Ukoha PO. and Ejikeme PM. (2010). Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods. *African journal of pure and applied chemistry*; 4(9): 206-212.

Okonogi T, Hattori Z, Ogiso A and Mitsui S. (1979), Detoxification by permisiontanin of snake venom and bacterial toxins. *Toxicon J*ournal; 17:524–527.

Paulchamy C. (2010), Pharmacological perspectives of snake venoms from Viperidae family. *The Internet Journal of Pharmacology.* Volume 8(2): 32

Santosh RF, Shivaji PG and Phcog Mag (2004).Preliminary screening of herbal plant extracts for anti-venom activity against common sea snake, *Indian Journal of Biochemistry.*16,56 – 60.

Saxena,Y., Saxena,M., Nema,R., Singh, D., and Gupta, A. (2013). Phytochemistry of medicinal plant. *Journal of pharmacognosy and phytochemistry. 1(6): 168-182.*

Serrano, S., Luis, J., Ayeb, M. E.l, and Marrakchi, N. (2009). Snake Venom Peptides: Promising Molecules with Anti-Tumor Effects. *Bioactive Food Peptides in Health andDisease*; 219-238.https://doi.org/10.5772/3318

Soladoye, M. O. and Chukwuma, E. C. (2012). "Quantitative Phytochemicals profile of the leaves of CissuspopulneaGuill. andPerr. (Vitaceae) – an important medicinal plant in central Nigeria". *Archives of Applied Science Research*, 4(1): 200-206.

Solomon M, Petes KJ. and Azage T (2004), Effect of supplementation with foliage of selected multipurpose trees, their mixtures or wheat bran and feed intake, plasma enzyme activities, live weight and scrotal surcumfrances gain in Menze sheep. *Journal of Livestock Production Science*; 89: 253-264.

Theakston RDG, Warrell DA, Griffiths E (2003): Report of a WHO workshop on the standardization and control of antivenoms. *Toxicon* 41: 41–557.

Ushanandini, S., Nagaraju, S., Harish K.K, Vedavathi, M, Machiah, D.K, Kemparaju K (2006). The anti-snake venom properties of *Tamarindusindica* seed extract. *PhytotherResourse Journal* 20(10): 851-858

Valevan JA, Segovia-Cruz FS, Rojas-Hualpa JM, Martins-de-Souza d. and Ponce-Soto LA. (2015), Functional and structural characterization of a new serine protease with thrombin-like activity TLBan from Bothropsandianus(Andean lancehead) snake venom. *Toxicon*; 59: 231-240

Vidyadhar S, Saidulu M, Gopal TK, Chamundeeswari D, Rao U. and Banji D. (2010), In vitro anthelmintic activity of the whole plant of *Enicostemma littorale* by using various extracts. *International journal of applied biology and pharmaceutical technology*; 1(3): 1119-1125

Vineetha M., Bhavya J. and Sunil S. (2017). Biochemical and pharmacological neutraliuzation of Indian saw scaled viper snake venom by C. parviflorum extxracts. *Indian journal of Biochemistry*; vol 54. pp. 173-185