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Characterization and Evaluation of the Effects of *Indigofera pulchra, Aristolochia albida* and *Andrographis paniculata* Leaves Extract Phenolics against the Activity of *Naja nigricollis* and *Echis ocellatus* Snake Venoms

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

With the 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 remote areas. This research was aimed at characterization and evaluation of the effects of *Indigofera pulchra, Aristolochia albida* and *Andrographis paniculata* leaves extract phenolics against the activity of *Naja nigricollis* and *Echis ocellatus* snake venoms. The plants samples were extracted using chloroform, after which a qualitative and quantitative phytochemical analysis was done, followed by characterization analyses (GC-MS and FTIR). Preperatory and analytical tin layer chromatography analyses was carried out on all the extracts, flavonoids and tannins fractions were isolated, using garlic and tannic acids as standards. In-vitro inhibition analyses of the partially purified phenolics was done to ascertain the effects to the isolated phenolic fractions against the two selected crude snake venoms. The plant extracts characterization done revealed that all the three extracts contain

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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 revealed 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 revealed that these plants' phenolic fractions have effects on the two snake venoms and can help in the management and treatment of snake bite.

Keywords: Indigofera pulchra; snake venoms; antiserum; phenolic extracts.

1. INTRODUCTION

Plants have been for long seen and exploited as potential source of medical agents and can be traditionally used to treat many diseases and infections especially infectious diseases including diarrhea, fever, cold and numerous infections [1]. Plants can however be also used in the treatment and management of zoonotic hazards such as bites from snakes, bees, scorpions 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 [2].

These plant compounds, with medicinal capabilities are known and referred to as Phytochemicals. These are also referred to as phyto-metabolites. Which are usually produce by plants that aids them in depending or fighting against competitors, predators, or pathogens [3]. 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 [4]. 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 [5]. Phytochemicals are usually confused with phytonutrients, but phytochemicals include plants compounds that are useful and those that are 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 [6]. Therefore the difference between phytochemicals and phytonutrients is quite essential, as not all phytochemicals are beneficial [7]. These chemicals are normally accumulated and concentrated in different parts of the plant, such as in the fruits, flowers, leaves, stem or roots. Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues and its level vary from plant to plant depending upon the variety, processing, cooking and grooving condition [8].

Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acid and chlorophylls [9]. While the secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumins, saponins, phenolics and glucosides [10]. Literature survey indicate that phenolics are the most numerous and structurally diverse plant phytoconstituent [11]. 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 [12]. 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 [13]. 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 [14]. Efforts are continuously being made to develop alternative treatment strategy from medicinal plants [15]. This research was focused on evaluating the effect of *Indigofera pulchra, Aristolochia albida* and *Andrographis paniculata* leaves extract fractions against the activity of *Naja nigricollis* and *Echis Ocellatus* snake venoms.

2. MATERIALS AND METHODS

2.1 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.

2.2 Snake Venom Sample Collection

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

2.3 Preparation and Treatment of Plant Samples

The leaves were surfaced sterilized, air-dried under shade, ground to powder using mortar and pestle and stored in an air-tight container as described by Lakache, [16].

2.4 Plant Material Extraction Protocol (Maceration)

This was carried out according to the method of Kumar, [17], using chloroform as the extraction solvent. The fine powder of leaves (290 g each) was 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), weighed and stored in sterile air-tight containers.

2.5 Phytochemical Screening

Quantitative and qualitative phytochemical analyses were carried out using standard procedures as described by Velavan, [18].

2.6 Thin Layer Chromatography (TLC)

Analytical Thin-layer Chromatography Thin Layer Chromatography was done according to the method of Lihua et al., [19]. 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 plate, 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 were air-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.

2.7 Preparative Thin-layer Chromatography

Pre-coated thick silica gel on glass TLC plates measuring 20 cm × 20 cm 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. 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 moved above the origin to the distance the solvent moved above the origin [14].

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.

2.8 Extract Evaluation Analysis

Gas chromatography-mass spectrometry (GC-MS) and fourier transform infrared spectroscopy (FTIR) analysis were carried out using standard procedures as described by Soladoye, [20] and Saxena, [21] respectively.

2.9 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 hadsimilar compounds with the standard [14].

2.10 Venom Protein Inhibition Studies

This is carried out using standard procedures as described by Nwune, [22], were the total protein concentration of the crude venom was tested prior and after addition of the partially purified phenolics.

2.11 Statistical Analysis

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

3. RESULTS

Result for the plants sample extraction of all the three plants carried out, revealed the physical properties and percentage yield of 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 Tables 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, as shown in Fig. 4a, 4b and 4c. 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.

4. DISCUSSION

Snake envenomation has for long, been an issue of serious economic and medical importance. And it happens that the only medical treatment for snake bite is by parenteral 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 and its standardization are found to be expensive, difficult and require ideal storage conditions [23]; 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, have some anti-snake venom capabilities [24].

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	-	ND
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	_	ND
13	Phlobatannins	+	ND
14	Flavonols/flavones	_	ND
15	Coumarins	_	ND
16	Quinones	_	ND
17	Resins	+	ND
18	Amino acids	+	ND
19	Chalcones	+	ND
20	Vitamin A	_	ND
21	Vitamin D	+	ND
22	Acidic compound	+	ND

Key: + = Presence - = Absence, Results are presented as mean ± standard deviation, ND = Not Detected

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	+	ND
12	Phlobatannins	-	ND
13	Flavanols and flavones	+	ND
14	Coumarins	+	ND
15	Quinines	-	ND
16	Chalcones	-	ND
17	Steroids	+	ND
18	Vitamin A	_	ND
19	Vitamin D	-	ND
20	Acidic compound	+	ND
21	Resins	+	ND
22	Amino acids	-	ND

Key: + = Presence - = Absence, Results are presented as mean ± standard deviation, ND = Not Detected

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

Results are in mean ± standard deviation
ND = Not Detected

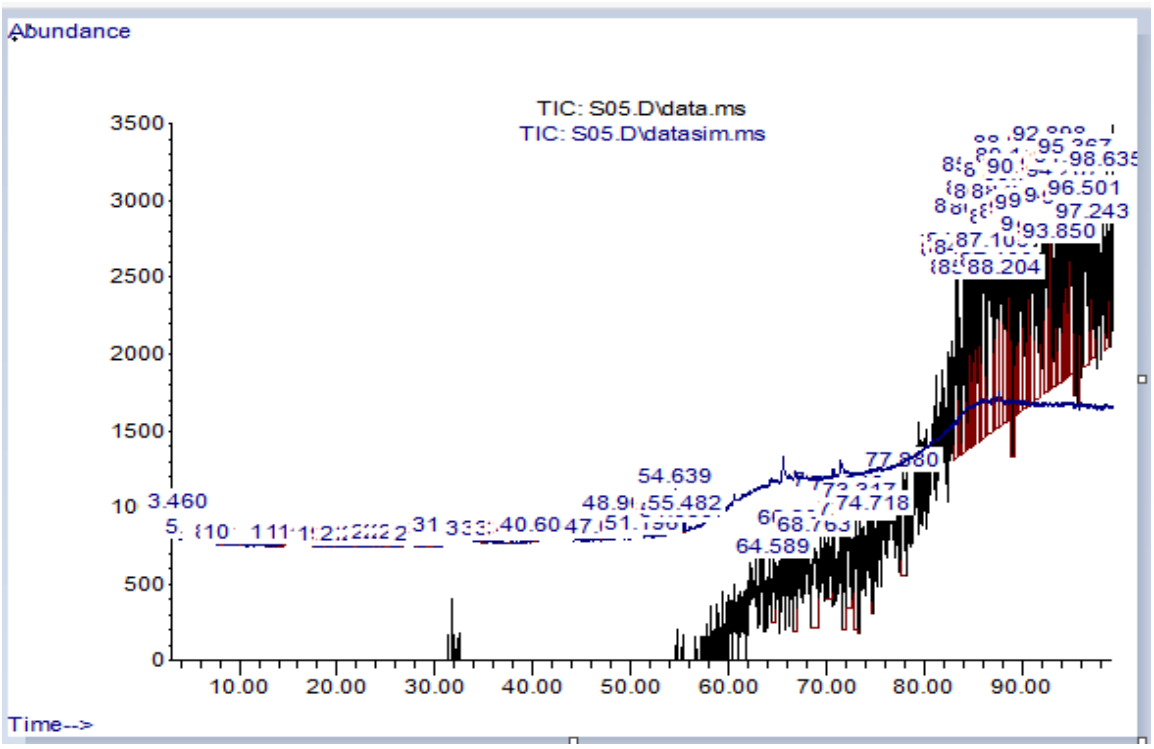


Fig. 1a. GC-MS analysis micrograph of *A. albida* chloroform leave extract

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

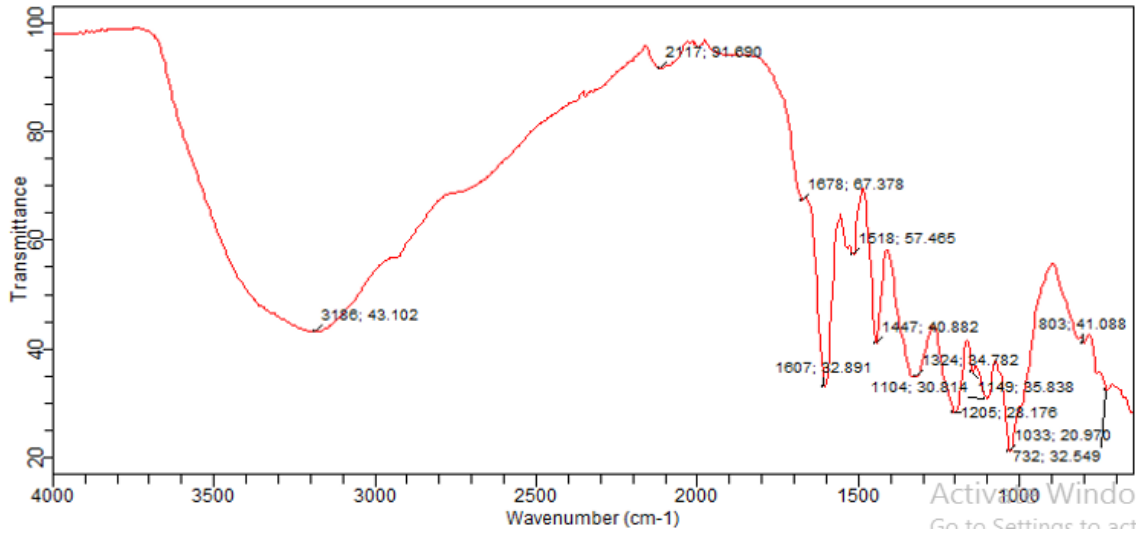


Fig. 1b. FTIR micrograph of *A. albida* chloroform extract

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	Ξ C − H Stretch	Alkynes
2	2200-2100	2117	C Ξ 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

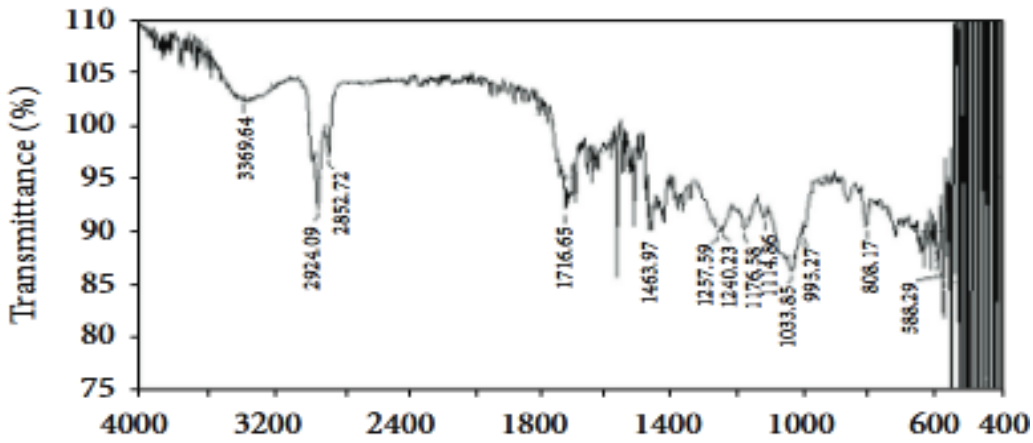


Fig. 2a. FTIR micrograph of *I. pulchra* chloroform extract

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

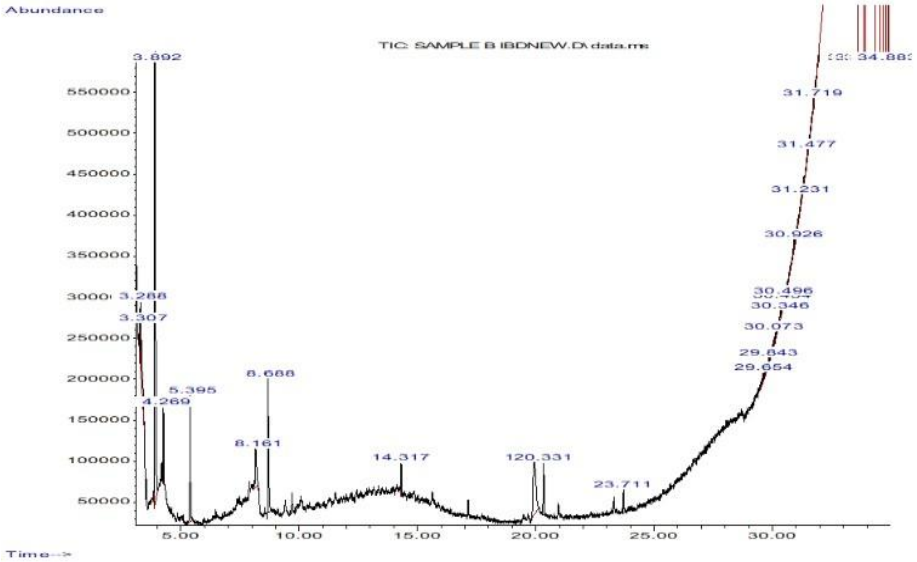


Fig. 2b. GC-MS micrograph of *I. pulchra* chloroform leave extract

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 ($C_{18}H_{33}NO$)	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($C_{16}H_{13}NO_4$)	7
5	89	1.67	N,N-Dimethylaminoethan ol ($C_4H_{11}NO$)	2
6	172	2.47	1,1,2-Trimethyl- 3,8,9-trioxa-bicyclo [4.2.1]nonane ($C_9H_{16}O_3$)	2
7	298	1.22	Methyl stearate ($C_{19}H_{38}O_2$)	2
9	193	2.11	1-(4-Methoxy-3- methylphenyl)-2- methylpropan-2-amine ($C_{12}H_{19}NO$)	4
10	126	4.16	Maltol ($C_6H_6O_3$)	
11	180	2.38	Theobromine ($C_7H_8N_4O_2$)	2
12	214	1.15	Dodecanoic acid, methyl ester ($C_{13}H_{26}O_2$)	2
13	270	1.35	Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$)	4

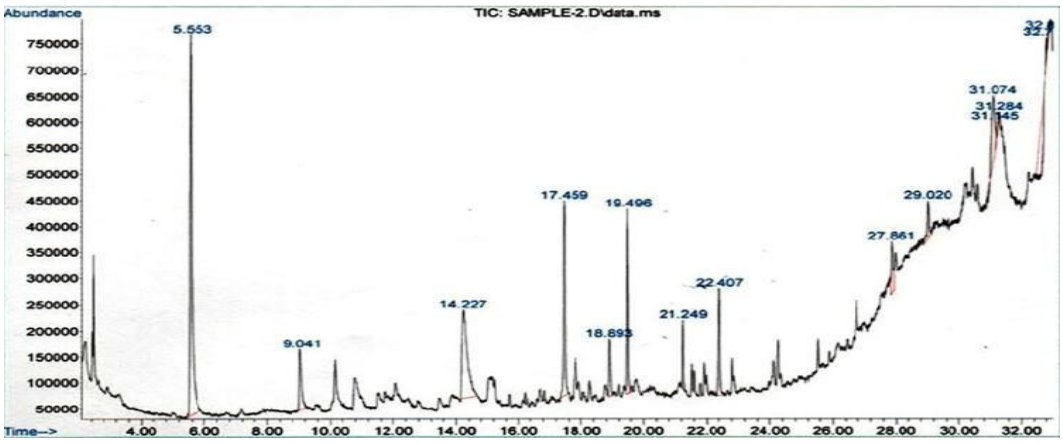


Fig. 3a. GC-MS micrograph of *A. paniculata* chloroform leave extract

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

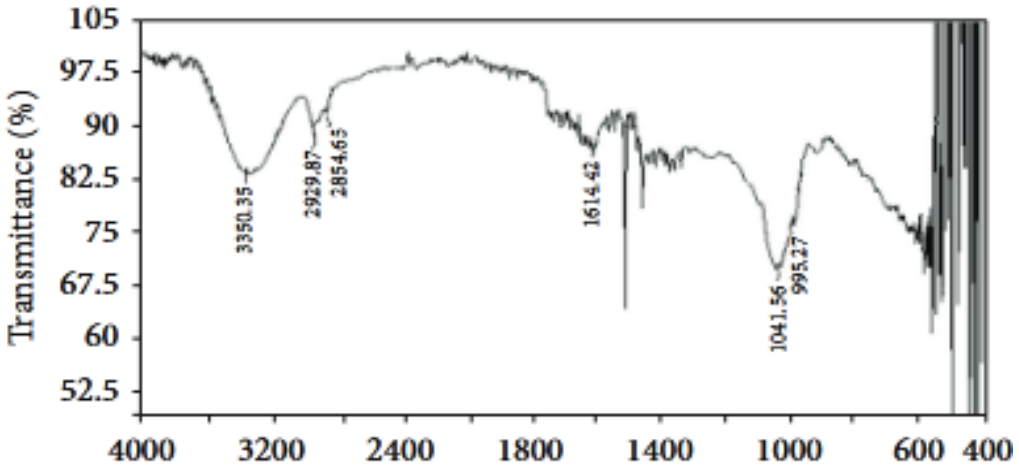


Fig. 3b. FTIR micrograph of *A. paniculata* chloroform extract

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)
	Garlic Acid		292.5
Standards	Tannic Acid		310
		1	**290[1]***
		2	**305[2]***
	A. albida	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*

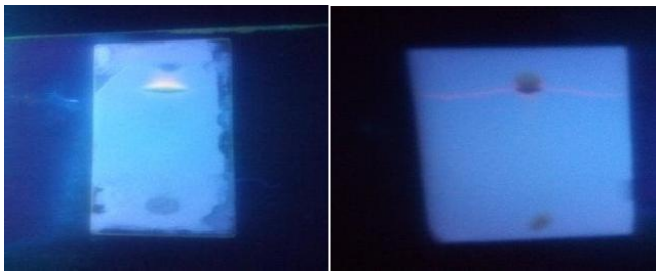


Fig. 4a. TLC plates for standard (garlic acid and tannic acid)

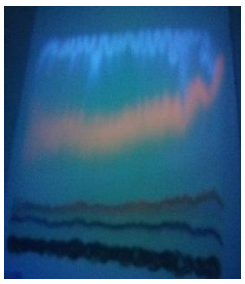
A.  B.  C. 

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

1.  2.  3. 

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

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

In this study the efficiency of the phenolic extracts of *A. albida, A. paniculata* and *I. pulchra* were 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 [25]. 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, which has been reported to have some anti-snake venom potentials [26]. 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 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. [27], where he stated that 'phytochemicals due inhibits venom 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 receptors Evans et al., [28].

Gomes et al. [24] reported that the herbal constit- uents are active against snake envenomation including among others; alkaloids, steroids, tannins, flavonoids and terpenoids. Okonogi et al. [29] 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. [28] reported that tannins precipitate proteins and form dark-coloured complexes with metals such as iron. Similar studies was conducted by Ushanandini et al. [30,31], which indicated that *Tamarind* seed extract inhibited the activity of snake venom proteins like; PLA_2, protease, hyaluronidase, l- amino acid oxidase and 5'-nucleotidase in a dose-dependent manner.

5. CONCLUSION

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

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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