ANTHELMINTIC ACTIVITIES OF HEXACOSA-9, 11-DIENOIC ACID AND 3-HYDROXYURS-12-EN-28-OIC ACID ISOLATED FROM *SPERMACOCE VERTICILLATA*

ABSTRACT

The phytochemical screening, anthelmintics, and characterization of *Spermacoce verticillata* crude ethanolic extracts were carried out using standard methods. The research is aimed at investigating the in-vitro anthelmintic activity of *Spermacoce verticillata* and *Cochlospermum tinctorium* plants for possible active components. The results of phytochemical screening showed the presence of Alkaloids, Flavonoids, Tannin, Phenol, Fatty acid and Cardiac glycoside, Terpenoid and Saponin. The result of the anthelmintic activities showed that *Spermacoce verticillata* have paralysis time ranging from 25.9 -71.6 mins with death times ranging from 39 - 90.1 mins at concentrations range between 25 - 100 mg/cm^3 The results of Chromatographic and Spectroscopic Analyses using TLC, CCH, IR, NMR GC-MS of *c* leads to the Isolation of two compounds namely Hexacosa-9,11-dienoic acid (fatty acid) and 3-hydroxyurs-12-en-28-oic acid (Pentacyclic triterpenoid) with paralysis/death time at concentration of 50 mg/cm^3 of 22.5 mins and 36.3 mins for the fatty acid and 13.2 mins and 20.1 mins for the pentacyclic triterpenoid. The results were compared with standard drug (Albendazole) which showed higher activity than the crude extracts but lower than the isolated compounds. The results of this work therefore confirmed the traditional claim of using the plant for the treatment of helminthiasis disease.

Keywords: Anthelmintics, *Spermacoce verticillata, Cochlospermum tinctorium,* Phytochemical screening

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INTRODUCTION

The world's population is concentrated in developing and impoverished areas, susceptible to different kinds of diseases (Tariq and

Tantry, 2012). Among the diseases common to impoverished developing areas includes' helminthiasis. This is a parasitic worm (helminth) infection transmitted to human or other animal through air, food or water. In humans helminth infections result in elephantiasis, schistosomiasis river blindness, guinea worm, hook worm etc. Helminths have the capacity to steal vital body nutrients in an attempt to survive and at the same time develop resistance to anthelmintic drugs. (Tariq, *et al.,* 2008). The resistance developed by helminthes towards drugs, calls for consistent anthhelmintinc investigation. These could be done with the view of providing suitable alternatives; as a result, natural products from medicinal plants are screened for anthelmintic activities. In this anthelmintic research two medicinal plants were targeted for investigated for efficacy in helminthiasis. The plants include *Spermacoce verticillata* and *Cochlospermum tinctorium*. These plants are traditionally used in various parts of Nigeria for the treatment of Schistosomiasis. According to the world health organization (WHO) Medicinal plants are plants that contains properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs (WHO 2008). Therefore these plants being researched are investigated for phytochemical compounds strategic to curing helminth infections. Any bioactive photochemical compound isolated could be developed into anthelmintic drugs which could be used to replace synthetic anthelmintic drugs. (Tariq *et al.,* 2008). The aim of this experiment is to investigate the in-vitro anthelmintic activity of *Spermacoce verticillata,* isolate and characterized the possible active components from the most bioactive plant.

EXPERIMENTAL

Materials and Methods:

Analytical grade chemicals and solvents (Ethanol, Methanol, Ethylacetate, n-Hexane, Petroleum ether, Potassium Iodide, Macuric chloride, Iodine and Diethyl-ether) were purchased for this research work. *Terrestris lumbricoides* and *Taenia saginata* were collected from a swampy rice farm by river Kaduna at Unguwar Maigero and Kaduna Zango abattoir respectively, Kaduna State.

Plants Sampling and Authentication

The targeted medicinal plant *Spermacoce verticillata* and *Cochlospermum tinctorium* were collected from Kafom in Tudun Wada District of Zangon Kataf Local The plants were identified and authenticated Mal Musa Gallah of the herbarium unit in the department

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of Biological Sciences, Ahmadu Bello University Zaria and assigned Herbarium No. 3154 and 9731 respectively. The shoots of the plants were cut off and properly washed using tap water and rinsed with distilled water before air drying under shade. The dried plant sample material was pulverized into powder and stored in a clean polyethylene bags at ambient temperature for further analysis.

Extraction

A portion (50 g) of pulverized powdered medicinal plant sample was percolated in a percolator using 350 cm^3 of ethanol for 2 weeks. The extract was filtered and evaporated to dryness at 40^oC using rotary evaporator. The crude extract obtained was allowed to dry and weigh to constant weight and the percentage yield calculated.

Phytochemical Screening

The extract was screened for the presence of alkaloids, flavonoids, glycosides, cardiac glycosides, anthroquinone, tannins and saponins according to standard protocol (Trease and Evans, 1989; Sofowora, 1993).

Anthelmintic Activities of Crude Extracts

The anthelmintic activity test was performed according to Ghosh *et al.,* 2005 on Adult earthworms (*Terrestris lumbricoides*), *Taenia saginata* and *Taenia solium* but with modification as adopted by Omale and Ubimago 2014. Equally, Albendazole, and Piperazine, were used as the standard reference drugs. Different concentrations of 25,50 and 100 mg/cm^3 were prepared using normal saline (0.9% NaCl) for both the crude extracts and the standard drugs. Each of the prepared solution was separately poured into differently labeled petri dishes with one of the petri dish left with only normal saline to serve as control. Five adult earthworms were carefully transferred into each labeled petri dish room temperature and a stop-watch was used to monitor the behavior of the worms. The time for paralysis was noted down when no movement of any sort could be observed except when the earthworms were shaken vigorously. The time of death for the worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50^oC). The experiment was repeated trice and a statistical mean was obtained. The same procedure was repeated for *Taenia saginata* and *Taenia solium* using 100 cm^3 beakers. Based on the results obtained the most bioactive crude extract selected for further analysis.

Large Scale Individual Extraction of *S. Verticillata.*

A portion (250 g) of dry plant sample will be moistened with about

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500 cm^3 of ethanol and allowed to stand for 2 weeks in a well closed container. The solution was filtered using Whatman no. 1 filter paper. The marc obtained after filtration was re-percolated for another 1 week and filtered. The combined filtrate was evaporated in a rotary evaporator at 40°C. The crude extract was allow cooling then weighed and was stored in the refrigerator before the activity guided column chromatography.

Thin Layer Chromatography of Crude Ethanolic extract:

Standard thin layer chromatographic plates coated with silica gel mesh 40 – 60 were used for the TLC process of the most bioactive (anthelmintic) crude extract. The TLC plates was initially activated at a temperature of 110°C for 30 mins and allowed to cool. Using a pencil a line was drawn 1 cm from the base and plate was spotted with the chromatogram using capillary tube. The spotted plates were developed separately using, solvents of increasing polarity in a developing tank. After development, the solvent (mobile phase) was allowed to evaporated and constituent spots identified by iodine vapour (Mayo *et al;* 2011). Solvent mixtures that give good resolutions were selected for column chromatography.

Column Chromatography of Extract

The glass column was thoroughly washed with detergent, rinsed with distilled water and solvent mixtures to be used as the mobile phase. It was allowed to dry and plug of wool was pushed down the bottom of a column and the column clamped on a stand. A 600 g mass of silica gel (70-110 mesh) was weighed and activated in an oven at 100°C for 1 hour then allowed to cool at room temperature in a desiccator for 30 minutes. Slurry of the silica gel (SiO_2) was prepared with chosen solvent mixture of solvents that gave good resolution during tin layer chromatography. The slurry was then gently transferred down the column with gradual release of control tap for the free flow of the solvent for the silica gel to be well packed and avoid cracking. A concentrated pre-absorbed (15 g) was carefully placed at the top of loaded sand and covered with sand. The column was eluted with the solvent mixture and collected in fractions of 100 cm^3. Each fraction collected were subjected to rigorous TLC using different mixture of solvents and the results obtained were used to pooled similar fractions with same RF value together and labeled. The pooled fractions were evaporated at 40^{0}C using rotary evaporator. Activity test were conducted using the same procedure and the most potent fractions were further subjected to column chromatography leading to the isolation of three different

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compounds. Bioactivity of the isolated compounds showed very promising results. (Mayo *et al.,* 2011)

Results and Discussion

The results of phytochemical screening, anthelmintic activities of the crude extracts, isolation and characterization of the most potent crude extracts were presented in Tables 1 – 5 respectively. The results of phytochemical screening showed the presence of Alkaloids, Cardiac glycosides, Flavonoids, Phenols, Saponins and Terpenoids. Presence of these secondary metabolites proved the potentialities of the plant for medicinal purposes. Research reports have indicated that phenols and flavonoids have anti-cancer and anti-oxidant properties. (Venugopal and Liu, 2012), Cardiac glycoside can be used for cardiac therapy (Wilcox *et al.,* 2007), Alkaloids for possible anti-malarial activity. (Pascal *et al.,* 2013). The presence of Tannins further proved that the plants can be used for anthelmintic activities (Horvath, 1991).

Table 1: Results of Phytochemical Screening of Crude Ethanolic extracts.

S/N	EXPERIMENT / TEST	*C. TINCTORIUM*	*S. VERTICELLATA*
1	Alkaloids	(+)	(+)
2	Cardiac Glycoside	(++)	(+)
4	Flavonoid	(+)	(++)
5	Phenol	(++)	(+)
6	Saponins	(-)	(+)
7	Tanins	(+)	(++)
8	Terpenoids	(-)	(++)

Key: + Presence ++ Presence at higher concentration

The results of anthelmintic activities of the crude ethanolic extracts showed that *Spermacoce verticillata* has relatively higher actitity against the test warms with paralysis time of 25.9 mins and death time of 39.0 mins at 100 mg/g (Table 2). However, as the concentration of the crude extracts decreases the activity also decreases, indicating that the activity of the crude extracts of the plant was concentration dependent. The activities of the crude extracts ranged from 25.9 to 72.0 for paralysis time and 39.0 to 91.9 for death time (Table 2). These activities were compared with the standard drugs which show a slightly higher activity than the two crude extracts at the same concentration. The anthelmintic activities displayed by these two crude extracts could be attributed to the presence of phytocompounds such as tannins, saponins and terpenoids. Tannins and Terpenoids have the ability to bind free proteins in the gastrointestinal or glycoprotein on

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the cuticle of the parasite and may cause death. (Anthanasiadou *et al.*, 2001). The presence of saponins in the crude as saponins is known cause parallel irritation of mucus membrane which could lead to death of the parasite (Mai, *et al.*, 2010). Phenols also the ability to form phenolic complexes capable of interfacing with energy generation in helminth parasite thereby by causing uncoupling oxidative phosphorylation that directly affects energy generation in the helminth parasite. (Hemamalini, *et al.*, 2013).

The crude extract of *Spermacoce verticillata* was subjected to chromatographic separations (TLC and Column) that leads to the isolation of two compounds whose spectroscopic analyses using IR, NMR and GC-MS showed that they are Hexacosa-9,11-dienoic acid and 3-Hydroxyurs-12-en-28-oic acid labeled A and B respectively.

Table 2: Results of Anthelmintic Activity of the Crude Extracts and reference standard

Plant Crude Extract	Concentration (mg/cm^3)	Paralysis time (mins)	Death time (mins)	Average Paralysis	Average Death
Albendazole (Reference Standard)	100	23.1 25.1 24.6 24.0	31.5 33.2 32.1 32.7	24.2	32.4
	50	47.9 48.8 50.5 49.2	60.8 61.8 60.0 62.3	49.1	61.2
	25	70.4 70.9 71.9 71.1	84.7 85.2 83.5 84.2	71.1	84.4
Cochlospermum tinctorium	100	34.2 36.1 35.5 35.2	46.3 47.3 48.0 47.1	35.3	47.2
	50	56.9 56.1 58.0 57.2	74.1 75.2 73.9 74.9	57.0	74.5
	25	71.9 71.4	92.5 91.2	72.0	91.9

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		72.2 72.5	92.1 91.9		
Spermacoce verticillata	100	26.7 24.8 26.2 25.9	38.9 37.9 40.3 39.0	25.9	39.0
	50	50.2 49.5 50.5 50.0	66.4 65.9 66.8 66.3	50.1	66.4
	25	71.2 72.2 71.0 72.1	91.1 89.8 89.4 90.0	71.6	90.1

Structural Elucidation and Result Discussion (Compound A):

The GC-MS spectra showed the molecular ion at *m/z* 392 and the IR spectrum showed a broad hydroxyl (OH) group at 3417.24 cm^{-1} and a strong carbonyl (C=O) absorption at 1701.06 cm^{-1} (Dudley and Ian 2006). Also the absorption peaks at 2915.42 and 2848.17 cm^{-1} are due to $C\text{-}H_{str}$ of methyl and methylene groups.

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The ^{1}H-NMR and ^{13}C-NMR alongside 2D-^{1}H, ^{1}H-COSY, HSQC and HMBC were tabulated in Table 4. The H-NMR data showed 26 different protons as indicated in the table below.

Table 3: ^{1}H-NMR and ^{13}C-NMR, ^{1}H,^{1}H-COSYand HMBC Data of Compound A

Position	^{13}C-NMR	^{1}H-NMR	^{1}H, ^{1}H-COSY	HMBC
1	179.96 (s)	-		
2	37.29 (t)	2.390		
3	27.16 (t)	2.019		C(2)
4	27.07 (t)	2.004	H-C(5)	C(2),C(5)
5	29.35 (t)	1.303	H-C(6)	C(4),C(7)
6	29.65 (t)	1.303	H-C(7)	C(7)
7	34.06 (t)	2.321	H-C(8), H-C(6)	
8	128.85 (d)	5.342	H-C(7)	
9	130.02 (d)	5.371	H-C(10)	

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10	39.37 (t)	2.358	H-C(11)	
11	129.73 (d)	5.345	H-C(10)	
12	130.91 (d)	5.386	H-C(13)	
13	34.06 (t)	2.321	H-C(12),H-C(14)	C(14), C(15)
14	24.80 (t)	1.668	H-C(14),H-C(15)	C(13),C(15),C(16)
15	29.77 (t)	1.303	H-C(14)	C(14), C(15)
16	29.33 (t)	1.303		
17	29.68 (t)	1.303		
18	29.37 (t)	1.303		
19	31.93 (t)	1.309	H-C(18), H-C(20)	
20	29.44 (t)	1.303		
21	29.25 (t)	1.303		C(23)
22	29.14 (t)	1.303		C(24),C(23)
23	29.07(t)	1.595	H-C(24), H-C(22)	C(22),C(24),C(21)
24	22.69 (t)	1.300	H-C(23)	C(26),C(23),(22)
25	22.62 (t)	1.300	H-C(26)	C(26),C(23)
26	14.11 (q)	0.862	H-C(25)	C(25)

The ^{13}C-NMR and DEPT spectra (400MHz) of compound A showed the presence of 26 Cs. The peak at (δ_C) 179.96 ppm is due to the presence of carbonyl carbon. The peaks at (δ_C) 128.85, 129.73, 130.02 and 130.91 are due to the presence of Methine Carbon at position 8, 11, 9 and 12 respectively. The ^{13}C-NMR also showed the presence of **21** Carbons up fields at (δ_C) values of 14.11 (C-26), 22.62 (C-25), 24.80 (C-14), 27.07 (C-4), 27.16 (C-3), 29.07 (C-23), 29.14 (C-22), 29.25 (C-21), 29.35 (C-5), 29.37 (C-18), 29.44 (C-20) 29.65 (C-6), 29.68 (C-17) and 29.77 (C-15) which all due to the presence of methelene carbons (CH_2). Presence of these large number of CH_2 and CH and absence of aromatic carbons suggests that the compound is a straight chain unsaturated hydrocarbon. Equally **4** slightly more de-shielded carbons appeared at (δ_C) 31.93 (C-19), 34.06 (C-7), 37.29 (C-2) and 39.37 (C-19) which are methelene Carbons. These methelene carbons that are appeared slightly more down field are due to the negative inductive effects exerted on them by the presence of doubles of methine carbons.

The ^{1}H-NMR spectrum ($400MH_Z$), Showed a signal at δ_H (0.862) due to methyl proton (H-26), also at δ_H 1.300 (H-25) is methelene proton attaché the methyl group as shown by COSY.

The chemical shift at δ_H 1.303 (H-29) is a high intensity multiplet of methylene envelope that spanned

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between δ_H1.305 – δ_H1.250. The shift δ_C29 on ^{13}C-NMR and DEPT Spectra shows the highest proportion (about 10) of carbon atoms; this implies that the envelope of multiplets at δ_H1.303 is a long chain of carbon. The ^{1}H, ^{1}H COSY Spectra reveals two groups of proton bonded to δ_H1.303, the first being a low intensity quintet δ_H1.668-1.595 then secondly another low intensity signal δ_H2.019-2.004. The bonding to a quintet signal at δ_H1.595 implies a methylene group flanked on both sides by other methylenes causing the quintet splitting of the signal. This signal on HSQC correlates with the signal on ^{13}C-NMR δ_C24 at position 14, while the low intensity of this signal correlates with the low proportion of these carbons relative to δ_C29 on the ^{13}C-NMR spectrum. The δ_H1.303 bonded to the multiplets signal δ_H2.004 on HSQC correlates with ^{13}C-NMR chemical shift signal at 27 (δ_C27), this bond signifies another phase of bonding for δ_C29.32 at position 5 and δ_C27.07 at position 4. The ^{1}H,^{1}H COSY spectra went further to show that the protons at δ_H1.595 C(24) was also bonded to δ_H2.358 C(13) protons. The δ_H2.358 signal is a multiplet spanning between δ_H 2.390 - δ_H2.321, this protons proximity to a deshielded double bond proton is obvious as they are bonded to a carbon adjacent to a double bond carbon (Dudley and Ian 2006). This is indeed obvious because the ^{1}H,^{1}H COSY spectra shows the link between these protons and the double bond protons at δ_H5.386. The HSQC spectra shows this protons bonded to a carbon at chemical shift value 34 (δ_C34). The ^{1}H-NMR showed the presence of four methine protons at δ_H 5.342 (H-8), 5.345 (H-11), 5.371 (H-9) and 5.386 (H-12) which supports the fact that the double bonds in this molecule are not conjugated. This is further confirmed by ^{1}H,^{1}H COSY spectra proving that the bonding pattern is commonly found in fatty acids. The assignment of double bond to C(8) and C(11) is based on the ^{1}H,^{1}H COSY and HMBC correlation with other carbons making up side chains from the double bonds as illustrated on table 5. subsequent bonding between C(14) and C(15) having δ_C29.77 and δ_H1.303 links the methylenic groups between δ_C29.77 - δ_C29.07 and δ_H1.303 forming the carbon chain joining C(15),C(16),C(17) etc, this correlates with ^{1}H,^{1}H COSY as well as HMBC result on table 5. Similarly consistent with this tabulated result is the bonding pattern on the other side of the double bond towards the COOH where C(8) δ_C 128.85 and δ_H 5.342 is bonded to methylenic C(7) δ_C 34.06 and δ_H2.321, further bonding of C(7) to methylenic C(6) at δ_C 29.65 and δ_H1.303 is followed by bonding to methylenic C(5),C(4),C(3), and C(2) as shown on table 5. Based on the spectral data derived from GC-MS, IR, ^{1}H-NMR,

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^{13}C-NMR the compound was confirmed to be Hexacos-9,11-diene-1-oic acid.

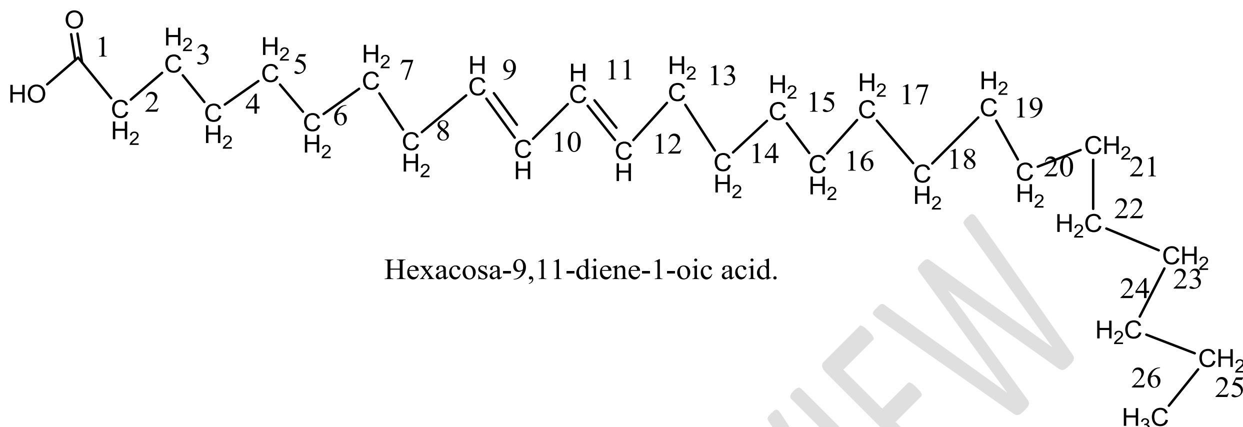

Figure 1. Structure of Hexacosa-9, 11-diene-1 acid

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Structural Elucidation of Compound B

Compound B was a white amorphous substance having a melting point of about 240.5-242.4. The compound showed a molecular ion ratio (*m/z*) of 456 when resolved in a GC-MS; this is in agreement with the molecular formula C_{30} H_{48} O_3. The infrared spectrum showed hydroxyl group at 3395.46 cm^{-1}, a carbonyl (C=O) at 1737.25 cm^{-1} and an olefinic group (C=C) at 1686 cm^{-1}. The characteristic C-H stretching for unsaturated and saturated functional groups were observed between 3000 cm^{-1} to 2922.06 cm^{-1} for this compound.

The compound showed a strong signal at 989.52 cm^{-1} within the finger print region which is a characteristic bending absorption of hydrogen attached to an unsaturated double bond (H-C=C). (Dudley and Ian 2006).

NMR and DEPT Spectra (400MH_Z, in DMSO) of this compound showed the presence of **30** carbon signals where **7** is for methyl groups (CH_3), **9** for methylene group (CH_2) and **9** methine group (CH) and **5** quaternary carbon atoms.

The ^{1}H-NMR spectrum gave peaks at δ_H 0.73 (H-24), 0.75 (H-25), 0.84 (H-25), 1.04 (H-23), 1.05 (H-27), 0.70 (H-29) and 0.86 (H-30) due to the presence of **7** methyl groups. This fact was supported by ^{1}H,^{1}H-COSY with protons at chemical shift δ_H 0.95 (C19) and δ_H 0.96 (C20) and

HMBC data for which H-C (29) has multiple bond connectivity with C(18), C(19) and C(20) while H-C(30) has multiple bond connectivity with C(19) C(20) and C(21). The data for 1D-^{1}H-NMR and ^{13}C-NMR alongside 2D-^{1}H, ^{1}H-COSY and HMBC for compound B are presented in Table 4.

Table 4: ^{1}H-NMR and ^{13}C-NMR, ^{1}H,^{1}H-COSYand HMBC Data of Compound (B)

Position	^{13}C-NMR	^{1}H-NMR	^{1}H, ^{1}H-COSY	HMBC
1	36.99 (t)	0.96	H-C(2)	C(2), C(3), C(10), C(9)
2	27.99 (t)	1.49	H-C(3), H-C(1)	
3	77.31 (d)	2.99	H-C(2)	C(2), C(4), C(24)
4	37.05 (s)			
5	55.25 (d)	0.70		
6	18.46 (t)	0.80	H-C(7)	
7	28.00 (t)	1.28	H-C(6)	C(5), C(6), C(8), C(25)
8	42.11 (s)			
9	47.48 (d)	1.48	H-C(11)	
10	37.15 (s)			
11	21.54 (t)	1.7	H-C(9)	
12	125.04 (d)	5.13		C(9), C(11), C(14),
13	138.67 (s)			
14	42.11 (s)			
15	27.45 (t)	0.84	H-C(16)	
16	28.73 (t)	1.31	H-C(15)	
17	47.30 (s)			
18	52.85 (t)	2.08	H-C(19)	C(17), C(19), C(20)
19	33.10 (d)	0.95	H-C(18)	C(18), C(20), C(21),C(29)
20	33.16 (d)	0.95	H-C(21)	C(19),C(21),C(30)
21	27.45 (t)	0.84	H-C(20), H-C(22)	
22	36.35 (t)	1.56	H-C(21)	C(17), C(20), C(21)
23	23.74 (q)	1.05		
24	17.39 (q)	0.73		
25	15.69 (q)	0.84		
26	17.48 (q)	0.75		
27	24.28 (q)	1.05		

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28	178.77(s)			
29	16.55 (q)	0.7	H-C(19)	
30	21.25 (q)	0.83	H-C(20)	

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The hydroxyl (OH) function at

C(3) is germinal to a proton atom corresponding to chemical shift (δ_H)2.99ppm which appeared as a broad triplet (br.t) and correlates with chemical shift (δ_C) 77.31ppm C(3). This is corroborated on ^1H, ^1H-COSY by correlation bond between H-C(2) and H-C(3) as well as HMBC correlation with C(1), C(5) and C(24).

The ^{13}C-NMR signal at 178.77 represents the carbonyl of the carboxylic acid group in the compound. The carboxylic group is predicted at (C-28) based on the fact that the carboxylic acid group has significant effect on the resonance values of C=C at positions 12 and 13. (Sun *et al.,* 2003). The presence of a carboxylic acid group (COOH) at positions C(14) (γ and δ to C(13) and C(12) respectively) alters the C=C Chemical shift to 129.1ppm and 134.1ppm for C(12) and C(13) respectively e.g. quinovic acid. (Sun *et al.,* 2003). According to Sun 'Except for those cases with COOH substituent in close proximity to a C(12)=C(13) bond, the chemical shifts of C(12) and C(13) are 125ppm and 138ppm respectively as in ursolic acid. The spectral data for GC-MS, IR and NMR proved that the compound is 3-Hydroxyurs-12-en-28-oic acid.

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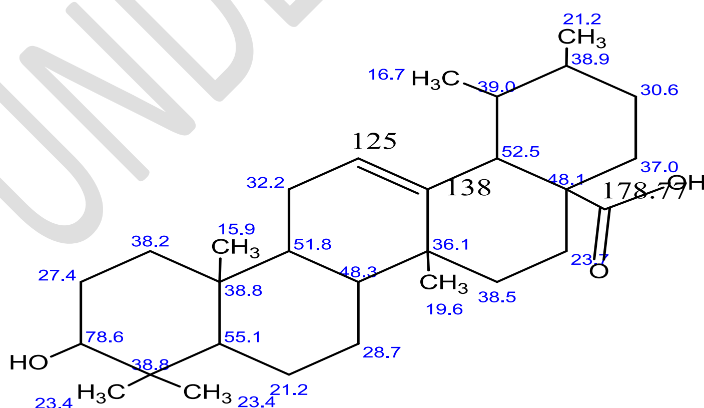

3-Hydroxyurs-12-en-28-oic acid.

Figure 2. Structure of hydroxyrus-12-en-28-oic acid

Anthelmintic Activities of the Isolated Compounds

A mean Paralysis time of 22.5min and 13.2min at concentration of 50mg/cm^3 for the fatty acid and pentacyclic triterpenoids respectively observed (Table 5) while, a mean death time of 36.3 min and 20.1 min at concentration of 50mg/cm^3 for the Fatty acid and pentacyclic triterpernoid respectively was observed (Table 5). In comparison with the Albendazole reference standard (table 4) having paralysis and Death time of 49.0 and 61.4 min respectively observed for a concentration of 50mg/cm^3, this showed the white amorphous crystal pentacyclic triterpenoids possess highest activity more than the fatty acid and the standard drugs. This could be due to the presence of more unsaturated reactive centers. It is important to note that the anthelmintic activities of both compounds are higher than that observed in the crude extract *Spermacoce verticillata* indicating that the synergistic effects other phytocompounds suppress the full potentials of the fatty acid and pentacyclic triterpenoids present in the plant.

Table 5: Anthelmintic Activities (*Spermacoce verticillata*) of the Isolated Compounds

Compound	Expt.Conc (mg/cm^3)	Paralysis Time (min)	Death time (min)	Average Paralysis time (min)	Average Death time (min)
Hexacosa -9,11-dienoic acid (Fatty Acid)	50	22.3 21.8 23.1 22.7	35.5 36.5 37.1 36.2	22.5	36.3
3-Hydroxyurs-12-en-28-oic acid. (Pentacyclic triterpenoids)	50	12.5 13.2 14.1 13.0	19.5 20.3 21.0 19.5	13.2	20.1

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Conclusion

Phytochemical screening of the two plants *Cochlospermum tinctorium* and *Spermacoce verticellata* reveals the presence of Alkaloids, Flavonoids, Tannins, Phenols, Terpenoids and Saponins. Anthelmintics activities of the two crude extract showed that the plants were active with *Spermacoce verticillata* having more activity against the test organisms. Chromatographic separation using solvent of increasing polarity leads to the isolation of two compounds. Spectroscopic analyses of the isolated compounds using IR, GC-MS and NMR reveal that, the isolated compounds are Hexacosa-9, 11-dienoic acid and 3-hydroxyurs-12-en-28-oic acid. This study showed that the plant contains molecules that could be used for the treatment of helminthiasis

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