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Anthraquinones and triterpenoids from *Senna siamea* (Fabaceae) Lam inhibit poliovirus activity

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The methanol extract of stem bark of *Senna siamea* (Fabaceae) was found to contain compounds with anti-poliovirus activity. Bioassay-guided fractionation of the extract resulted in the isolation and identification of six compounds; three triterpenoids- lupenone (1) lupeol (4) and betulinic acid (5); two antraquinones- chrysophanol (2) and physcion (3); and β- sitosterol glucoside (6). To evaluate the capacity of the compounds to inhibit the cytophatic effect of poliovirus in tissue culture, the reduction of viability of infected or uninfected cell cultures was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay. The selective indices of compounds with antiviral activity were determined by comparing the ratio of cytotoxic concentration at 50% to inhibitory concentration at 50% (CC_{50}/IC_{50}). Lupeol was the most active compound with IC_{50} value of 0.014 µg/mL but chrysophanol with IC_{50} 0.46 µg/mL was more selective with selective index (SI) of 32.1. The detection of pharmacologically active compounds in *S. siamea* extracts provides evidence that Nigerian ethno-medicines may be an important source of anti-poliovirus compounds. This study justifies ethnomedicinal uses of *Senna* as an anti-viral agent and also provides chemical entities that could lead to discovery and development of antiviral agents.

Key words: Antraquinones, triterpenoids, *Senna siamea,* MTT colorimetric assay, anti-poliovirus.

INTRODUCTION

Globally, there is concern on the post poliovirus era and the development of agents to combat or limit future outbreaks. In 2006, the National Research Council (NRC) stated that , it will be of a wide-reaching significance if new anti-viral compounds are developed (NRC, 2006). The reported anticipated three situations for polio antiviral

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drug use are as follows: for immunodeficient individuals chronically shedding poliovirus; for persons exposed to poliovirus (e.g. through unintentional laboratory exposure) (likely in conjunction with inactivated polio vaccine- IPV); for communities exposed to a circulating vaccine-derived poliovirus (cVDPV) in the post-eradication era. Successful development of an antiviral drug to prevent poliovirus transmission will require simultaneous attention to two major challenges: the first is to identify and optimize active agents, the second is to define the usage of the active agent (NRC, 2006).

Medicinal plants have been a constant source of safe and effective drugs, natural compounds have proved to be effective against a wide variety of viral diseases. In our previous work, *Senna siamea* (Fabaceae) Lam has been reported as component of antiviral remedies in the Nigerian ethnobotany. Preliminary anti-poliovirus activity of the crude methanol extract from its stem bark along with thirteen other medicinal plants has also been reported (Ajaiyeoba et al., 2006; Ogbole et al., 2013).

S. siamea is indigenous to the warm and wet tropical forests (Burkill, 1995). Other confirmed biological activities include; free radicals scavenging, antimicrobial and anti-malarial; Chrobisiamone A isolated from the leaves gave a good *in vitro* anti-plasmodial activity against parasite *Plasmodium falciparum* 3D7. Emodin and lupeol isolated from the ethyl acetate fraction were found to be the active principles responsible for the anti-plasmodial property of *S. siamea*, with IC_{50} values of 18.5 µM for emodin and 11.7 µM for lupeol (Ajaiyeoba et al., 2003; 2007; Kaur et al., 2006; Oshimi et al., 2008). In 2012, Hu and co-workers reported the isolation of antiviral chromones from the stem of *Cassia siamea*. The compounds showed both anti-tobacco mosaic virus (anti-TMV) and anti-HIV-1 activities. Cassiamin B present in the plant is an anti-tumour promoting and chemo-preventing agent (Sastry et al., 2003).

In our continuing search for antiviral agents from the Nigerian ethnomedicine, this report presents the isolation of anthraquinones and terpenoids with anti-poliovirus activity from *S. siamea.*

MATERIALS AND METHODS

Plant collection and extraction

Stem bark from *S siamea* were collected from the ground of University College Hospital (U.C.H), Ibadan, Nigeria. Voucher specimens were identified by Mr. O. A. Osiyemi at the herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan. Sample was deposited at the herbarium under voucher number FHI 108860. The stem bark was air-dried at room temperature (29-32°C) and pulverized. The dried powdered material (800 g) was extracted by maceration into methanol at RT (room temperature). (Obodozie et al., 2004).

Spectroscopic measurements

One dimensional (1D) NMR (^{1}H NMR, ^{13}C NMR, DEPT135 and DEPT90), and 2D NMR spectra, were recorded in deuterated solvents ($CDCl_3$ or in CD_3OD or pyridine) on Bruker AM-300, 400, or 500 MHz spectrometers (Bremen, Germany). Chemical shifts were measured in ppm (δ) and coupling constants (*J*) are given in Hz. UV spectra was taken in MeOH solution using a lambda 9UV/Vis./NIR Spectrometer (Perkin-Elmer, USA) IR spectra were recorded on $CHCl_3$ solutions on either a Perkin-Elmer 580 or Philips 9800 FTIR Spectrometer. Mass spectra/Electron Impact (EIMS) measurement was recorded on Varian MAT 312 double focusing spectrometer or on a Finnigan MAT 311 (Cambridge, England) with MASS PEC data system. Peak matching and field desorption (FD) experiments were performed on Finnigan MAT 312X mass spectrometer. Fast atom bombardment mass spectra (FAB MS) were recorded on Jeol HX 600 mass spectrometer.

Fractionation and isolation of bioactive compounds

As described in a previous study, dried powdered material was extracted into re-distilled methanol by maceration at room temperature; the extract was evaporated to dryness. Thereafter, the methanol extract (45 g) was re-suspended in MeOH: H_2O (70:30), the suspension was partitioned against *n*-hexane, $CHCl_3$, EtOAc to give their respective fractions. Both hexane and chloroform fractions from the crude methanol extract were active with 50% inhibitory concentration (IC_{50}) of 0.51 and 0.23 µg/mL, respectively (Ogbole et al., 2013).

In this study, the active *n*-hexane soluble fraction of *S. siamea* (10.1g) was subjected to column chromatography over silica gel (type 60, 70-230 mesh, E. Merck), column dimension (30 mm x 150 cm) eluted with step-gradient solvent system with Hex: EtOAc and $CHCL_3$: CH_3OH mixtures of increasing polarity. A total of 82 fractions were collected and monitored with thin layer chromatography (TLC, silica gel G, UV_{254}) seventy five of the fractions were eluted with Hex: EtOAc and while seven fractions were eluted with $CHCL_3$: CH_3OH. Fractions 19-21, eluted with 10% EtOAc in n-hexane (1L) precipitated to yield compound 1.

Compound 2 precipitated from fractions 24 and 25 that were eluted with 15% EtOAc in n-hexane. (Hex: EtOAc 85: 15, 500 mL). Thin layer chromatographic analysis of fractions 26-29 showed the presence of two compounds. The fractions were pooled and the resultant precipitate was solubilized in EtOAc and subjected to preparative thin layer chromatography (PTLC: silica gel (Merck, $60F_{254}$; thickness, 0.5 mm; Hex: EtOAc (95:5). Compound 3 (CH 28) was obtained from the PTLC. Compound 4, crystallized from fraction 31- 39, it was eluted with Hex 70%: EtOAc 30% (2L), while compound 5 precipitated from fractions 69-73 that was eluted with Hex 40%: EtOAc 60% (1.5L) while the solvent was been concentrated.

The chloroform soluble fraction of *C. siamea* was subjected to column chromatography on silica gel and eluted with $CHCl_3$: CH_3OH with stepwise increase in polarity; a total of 78 fractions were collected and monitored with TLC. Fractions 17 and 20, that were eluted with 100% $CHCl_3$ crystallized to give compound CC 19 R_F= 0.63 ($CHCl_3$: EtOAc, 95:5). Compound CC50 R_F = 0.63 ($CHCl_3$: EtOAc, 95:5) precipitated from the solution consisting of fractions 49-51, was eluted with $CHCl_3$:CH_3OH (70: 30, 1L).

Compound 6 R_F = 0.63 ($CHCl_3$: EtOAc, 95:5) precipitated out of the solution consisting of fractions 53-59, was also eluted with $CHCl_3$:CH_3OH (70: 30, 1L).

Deduced from TLC characteristics, compounds CH 32, CC 19 and CC 50 turned out to be similar compounds; this was further confirmed by spectrometric analysis. All compounds were analyzed using spectroscopic techniques (Obodozie et al., 2004).

Cells and virus culture

Poliovirus (Sabin Type1) was obtained from the WHO Polio

Laboratory, Department of Virology, University of Ibadan. Cytotoxic and antiviral parameters were determined in Human Rhabdomysarcoma (RD) cells (CDC, Atlanta, Georgia). Cells were grown in Eagle's MEM supplemented with 10% FBS, 100 units/mL of penicillin, 100 µg/mL of streptomycin, 2 mM L-glutamine, 0.07% $NaHCO_3$, and 1% non-essential amino acids and vitamin solution. The test medium used for cytotoxic assays and antiviral assays contained only 2 and 5% of the appropriate serum, respectively. Virus titers were determined by cytopathic effect in RD cell and were expressed as 50% tissue culture infective dose ($TCID_{50}$) per mL, viral stock was stored at −70°C until use.

Maximum non-toxic concentration test of extracts

This test was carried out to determine the maximum non-toxic concentration (MNTC) of compounds to RD cells in tissue culture. Serial ten-fold dilutions (100-0.00001 µg/mL of the compounds were made with maintenance medium. 96-wells plates previously seeded with monolayers of RD cells were treated with various concentrations of each extract. Plates were incubated at 37°C in a 5% CO_2 athmosphere for 72 h. Cell culture plates were then observed under the microscope (Inverted Phase Contrast Microscope, Olympus Objectives, Magnification 20 x) for cell death or destruction resulting from toxicity of plant extract. The minimum dilution of compounds with no toxic effect on the cells was referred to as maximum non-toxic concentration. The maximum non-toxic concentration (MNTC) for extracts/compounds was the dilution of extract at which, by microscopic examination, cells showed normal morphology and cell density in the presence of extracts when compared with the control cells grown without extract, and showed at least 95% of the optical density of the untreated cells. The maximum non-toxic test was used to determine the concentration of extract that will cause cell death in the absence of the virus while the cytotoxic assay (below) is used to determine what concentration results in the death of 50% of the cells

Cytotoxicity assay

Each compound was pre-solubilised at 37°C in dimethylsulphoxide (DMSO) to give a stock solution of 1 mg/mL. Serial two fold dilutions were made from stock solution using cell culture media to give working concentrations of 10-1000 µg/mL (the final concentration of DMSO did not exceed 0.5% which did affect cell growth). Confluent monolayers of RD cells were grown in 96 well tissue culture plates. Cells were incubated with various concentrations of the test compounds (10 - 1000 µg/mL) in quadruplicates at 37°C in a CO_2 environment. Thereafter, cell viability was examined microscopically for presence or otherwise of cytophatic effect (CPE). The second experiment was to determine the 50% cytotoxic concentration of each compound. Cells were incubated with various concentrations of the test compounds (10 µg to 1000 µg/mL) and cell viability was examined by ability of the cells to cleave the tetrazolium salt MTT [3-(4, 5- dimethylthiazol-2ol) 2, 5 diphenyltetrazoliumbromide), (Sigma Chem, USA), by the mitochondrial enzyme succinate dehydrogenase which develops a formazan blue color product and the procedure was followed as described earlier (Mosmann, 1983). Briefly, the supernatants were removed from the wells and 25 µL of an MTT solution (2 mg/mL in PBS) was added to each well. The plates were incubated for 1.5 h at 37°C, and 125 µL of DMSO was added to the wells to dissolve the MTT crystals. The plates were placed on a shaker for 15 min and the optical density was determined at 492 nm (OD492) on a multiwell spectrophotometer (Multiskan, MTX Lab, USA). The 50% cytotoxic concentration (CC_{50}) was defined as the test compound concentration required for reduction of cell viability by 50% and CC_{50} values were calculated by regression analysis.

Antiviral assay

Inhibition of cytopathic effect (CPE) assay was used for screening the antiviral activities of the compounds. To confluent RD cell monolayer in a 96-well plate, 100 $TCID_{50}$ (50% tissue culture-infective dose) virus suspension (50 µl) was added and left on bench for about an hour to allow virus adhesion. Thereafter, serial two-fold dilutions prepared from the maximum non-toxic dose of compounds were added in quadruplicate. As positive control, cells were infected with the same concentration of virus but without the addition of extract, and as a negative or cell control, only 2% MEM was added to the cells. The plates were incubated at 37°C in a humidified CO_2 atmosphere for 3 to 5 days. Cell viability was also determined by MTT assay. The concentration that reduced 50% of CPE with respect to the virus control was estimated from regression analysis of optical density data and was defined as the 50% inhibitory concentration (IC_{50}). SI was calculated from the ratio CC_{50}/IC_{50}. No control drug was used for this assay as currently there is no approved drug for the treatment of poliovirus infection.

Statistical analysis

Selective index, CC50 and IC_{50}

The CC_{50} and IC_{50} for each extract were calculated from concentration-effect-curves after linear regression analysis, using GraphPad® prism.

The SI was taken as the ratio of CC_{50} to IC_{50}. It is a comparison of the amount of a test compound that causes the inhibitory effect to the amount that causes death.

RESULTS

Fractionation and isolation of bioactive compounds

Compound 1 (CH 21) ($R_{F=}$0.58 silica gel G, Hex: EtOAc: 9.5:0.5) (0.318 g) was isolated by column chromatography, from hexane soluble fraction of *S. siamea*, a white crystalline powder, with melting point 168-169°C. It was positive to Liebermann Burchard test (Liebermann, 1885) giving a purplish red staining characteristic of triterpenoids. The EIMS (Varian MAT 312 double focusing spectrometer, Cambridge, England) showed the $[M]^+$ at m/z 424.2, consistent with the molecular formula $C_{30}H_{48}O$. By comparing NMR signals of proton and carbon spectrum of compound 1 with literature data (Table 1) (Hisham et al., 1995), the compound was identified as lupenone (Figure 1)

Compound 2 (CH 24) was isolated from hexane soluble fraction of *S. siamea* stem bark as yellow-orange needles with mp 202-205°C. It had an Rf = 0.71 (silica gel, Hexane: EtOAc 85:15) and intense yellow color in daylight, a yellow-orange color under UV365 light and a red color upon exposure to ammonia vapour. It was positive to Borntrager's test with a pink coloration, confirming it as an anthraquinone. The EIMS of compound 2 had $[M^+]$ at m/z 254 consistent with molecular formula $C_{15}H_{10}O_4$. On the basis of its spectroscopic (^{13}C-NMR) data, and by comparison to literature data (Coopoosamy and Magwa, 2006) the

Table 1. ^{13}C-NMR chemical shift assignments for Lupenone, lupeol and betulinic acid.

Position	Lupenone		Lupeol		Betulinic acid	
	^{13}C-NMR (δ)*,	^{13}C-NMR (δ) ($CDCl_3$, 400 MHz)	^{13}C-NMR (δ) *[a]	^{13}C-NMR (δ) ($CDCl_3$, 400 MHz)	^{13}C-NMR (δ) *[a]	^{13}C-NMR (δ) ($CDCl_3$, 400 MHz)
1	39.53	39.62	38.7	38.7	38.7	38.6
2	34.03	34.16	27.4	27.4	27.4	26.8
3	217.9	218.16	78.9	79.0	78.9	78.6
4	42.9	43.00	38.8	38.9	38.8	38.7
5	54.87	54.94	55.3	55.3	55.3	55.2
6	15.9	15.97	18.3	18.3	18.3	18.1
7	33.53	33.58	34.2	34.3	34.3	34.1
8	42.83	42.91	40.8	40.8	40.7	40.5
9	49.73	49.80	50.4	50.4	50.5	50.4
10	36.83	36.89	37.1	37.2	37.2	37.1
11	22.8	21.04	20.9	20.9	20.8	20.7
12	21.43	21.48	25.1	25.1	25.5	25.3
13	35.47	35.53	38.0	38.5	38.4	38.1
14	40.77	40.79	42.8	42.8	42.2	42.2
15	24.8	25.17	27.4	27.4	30.5	30.4
16	38.13	38.18	35.5	35.6	32.1	32.1
17	47.23	47.34	43.0	43.0	56.5	56.0
18	48.23	48.25	48.2	48.3	46.8	46.8
19	47.83	47.96	47.9	48.0	49.2	49.3
20	150.57	150.89	150.9	151.0	150.3	150.6
21	25.13	25.16	29.8	29.8	29.7	29.5
22	39.93	39.98	40.0	40.0	37.0	37.1
23	29.83	29.84	28.0	28.0	27.9	27.6
24	27.4	27.44	15.4	15.4	15.3	15.1
25	18	18.02	16.1	16.1	16.0	15.6
26	15.8	15.79	15.9	16.0	16.1	15.8
27	14.4	14.48	14.5	14.5	14.7	14.4
28	19.3	19.31	18.0	18.0	180.5	178.4
29	109.2	109.39	109.3	109.3	109.6	109.2
30	19.6	19.69	19.3	19.3	19.4	19.0

*Hisham et al., 1995; *[a]Sholichin et al., 1980.

compound was identified as chrysophanol (Figure 1 and Table 2).

Compound 3 (CH 28) was isolated from hexane soluble fraction of *S. siamea* bark as yellowish orange needles; mp 202-205°C, R_f = 0.58 (silica gel, Hexane: EtOAc 85:15). The TLC analysis confirmed it was a single compound which had intense yellow color in daylight, a yellow-orange color under UV365 and a red color upon exposure to ammonia vapour. The EI mass spectrum showed molecular ion at m/z [M^+] 284.1, supporting the formula $C_{16}H_{14}O_4$. The ^{13}C-NMR spectral assignment was made on basis of literature comparison (Wu et al., 2009) and identified the compound as physcion (Figure 1 and Table 2).

Compound 4 (CH 32, CC 19 and CC50), a white amorphous solid (402 mg), with melting point 213-216°C, was isolated by repeated column chromatography of the hexane and chloroform soluble fraction of *S. siamea* bark, Rf = 0.62 (silica gel, Hex:EtOAc, (9.5:0.5). It was positive to Liebermann Buchard test (purplish red staining) characteristic of triterpenes. The EIMS *m/z* was 426 [M^+]. Comparison of its ^{13}C-NMR data with literature (Sholichin et al., 1980) (Table 1), allowed the establishment of the structure of the compound as lupeol (Figure 1)

Compound 5 (CH 70) was isolated by repeated column chromatography of the hexane and chloroform soluble fraction of *S. siamea* bark extract. The R_f value analyzed by TLC using silica gel Hex: EtOAc, (9:1) was 0.54, with melting point 278-280°C, a reddish purple coloration in the presence of Libermann-Buchard reagent, suggested a triterpene. The EI mass spectrum showed molecular

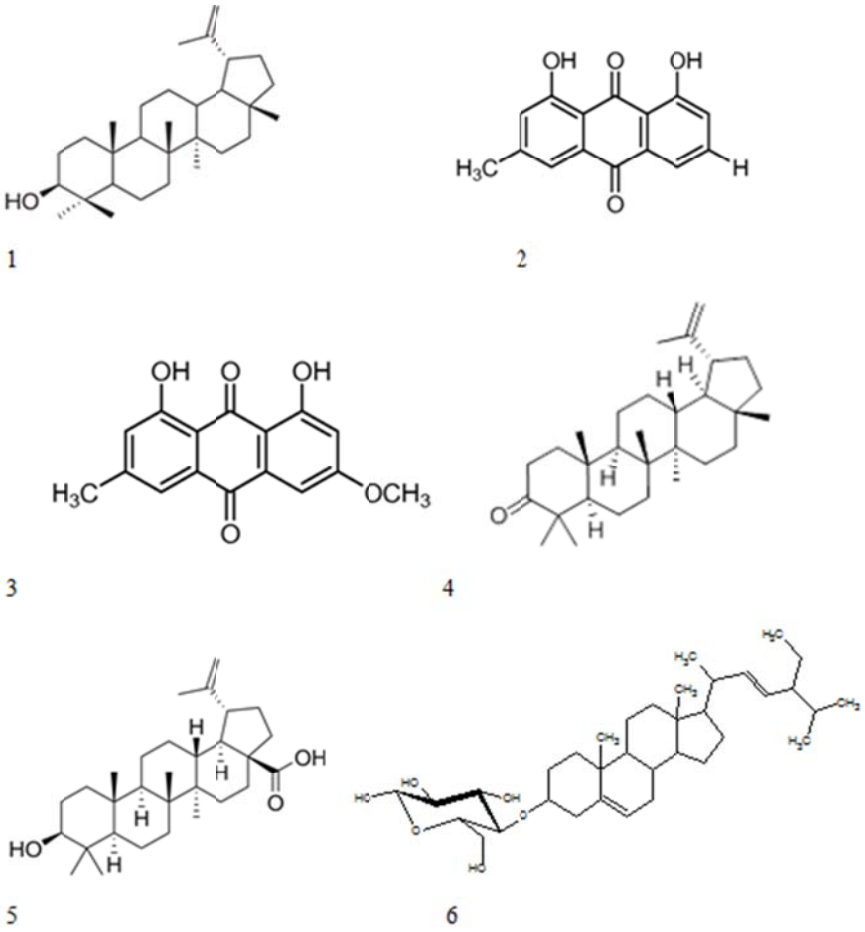

Figure 1. Structure of compound isolated from *Senna siamea* (Wu et al., 2009; Sholichin et al., 1980; Hisham et al., 1995). 1= Lupenone, 2 = Chrysophanol, 3 = Physcion, 4= Lupeol, 5= Betulinic acid, 6= β-sitosterol glucoside.

Table 2. ^{13}C-NMR chemical shift assignments for β-sitosterol-3-*O*-β-D-glucopyranoside.

Position	^{13}C-NMR (δ), ($CDCl_3$, 500 MHz)	^{13}C-NMR (δ)*	Multiplicity
1	39.39	39.50	CH_2
2	26.47	28.60	CH_2
3	78.65	80.90	CH
4	40.00	40.10	CH_2
5	140.97	142.10	C
6	121.93	121.7	CH
7	32.20	32.4	CH_2
8	32.10	32.3	CH
9	50.41	50.6	CH
10	32.67	32.7	C
11	21.32	21.6	CH_2
12	39.87	40.3	CH_2
13	42.53	42.8	C
14	56.97	57.1	CH
15	24.54	24.7	CH_2
16	28.56	27.0	CH_2

Table 2. Contd.

17	56.30	56.7	CH
18	12.01	12.1	CH_3
19	19.45	19.4	CH_3
20	36.42	36.5	CH
21	19.25	19.2	CH_3
22	34.27	34.6	CH_2
23	30.30	30.5	CH_2
24	46.11	49.5	CH
25	29.54	29.9	CH
26	19.99	20.0	CH_3
27	19.04	19.6	CH_3
28	23.45	23.7	CH_2
29	12.19	12.3	CH_3
C-1'	102.63	102.8	CH
C-2'	75.38	75.4	CH
C-3'	78.50	78.3	CH
C-4'	71.78	72.2	CH
C-5'	78.50	78.6	CH
C-6'	62.91	62.3	CH_2

*Mukhtar et al., 2002.

ion at m/z 223.1 was consistent with the molecular formula $C_{30}H_{48}O_3$. Comparing the NMR spectroscopic data with literature (Table 1) identified the compound as betulinic acid.

Compound 6 (CC59) precipitated from $CHCl_3$ soluble fraction, as a white amorphous powder, with melting point 278-280°C, R_F= 0.66 ($CHCl_3$/MeOH 4:1) and EIMS (rel. Int. %) m/z 414 (8.4), 396 (100), 382 (27.7), 275 (8.1), 255(26.3). The negative ion FAB MS showed the $[M-H]^+$ at m/z 575.02 consistent with the molecular formula $C_{35}H_{60}O_6$. The compound was identified as β-sitosterol-3-*O*-β-D-glucopyranoside by comparison with reported data (Mukhtar et al., 2002). The ^{13}C-NMR (C_5D_5N, 500 MHz) δ: (Table 3) spectral data obtained for compound CC59 when compared with literature showed the compound as β-sitosterol-3-*O*-β-D-glucopyranoside (Figure 1).

Cytotoxicity assay on rhabdomyosarcoma cells for isolated compounds

The cytotoxicity assay was analyzed to determine CC_{50} for isolated compounds; the result indicated that lupeol, one of the five compounds isolated from *S. siamea* was the most toxic compound with CC_{50} value of 0.40 µg/mL. Betulinic acid was the least toxic compound with CC_{50} of 50.46 µg/mL; physcion and chrysophanol had CC_{50} values of 38.04 and14.77 µg/mL, respectively (Table 4).

Antiviral activity of the isolated compounds

Inhibitory concentration (IC_{50} values) data obtained indicated that results obtained were concentration dependent and with agreeable correlation co-efficient (R^2) (Betancur-Galvis et al., 1999). Lupeol had the greatest inhibition with IC_{50} of 0.014 µg/mL. Chrysophanol and physcion had IC_{50} values of 0.46 and 1.57 µg/mL, respectively. Betulinic acid was the least active compound with IC_{50} values of 23.13 µg/mL as shown in Table 4.

Selective index

Selective index (SI) was calculated for the compounds, selectivity is classified as non-cytotoxic (SI >20), weak cytotoxic (SI ≥10) and a selectivity index higher than 10 is considered interesting for *in vitro* studies (Ondo et al., 2012) Chrysophanol and physcion were highly selective with SI of 32.3 and 24.3, respectively, betulinic acid had very low selectivity, lupeol which is the most active of the isolated compounds had SI of 29.4 (Table 4).

DISCUSSION

S. siamea belongs to the Family Fabaceae, subfamily Caesalpinioideae, which has been widely used in traditional medicine. Plants of this family have been extensively studied in traditional medicine and have been found to possess various biological activities (Junior et al., 2006).

Fractionation of the active chloroform and hexane soluble fractions of *S. siamea* led to the isolation of six compounds: lupeol, lupenone, betulinic acid, chrysophanol,

Table 3. ^{13}C-NMR chemical shift assignments for Chrysophanol and Physcion.

Carbon position	Chrysophanol		Physcion		Type of shift
	^{13}C-NMR (δ)*	^{13}C-NMR (δ), ($CDCl_3$, 500 MHz)	^{13}C-NMR (δ)*[a]	^{13}C-NMR (δ), ($CDCl_3$, 500 MHz)	
1	162.7	162.7	165.20	162.41	s
2	124.4	124.6	124.48	124.3	d
3	136.9	137.0	148.42	148.34	s
4	119.9	119.9	121.26	121.1	d
4’	133.3	133.3	133.23	134.0	s
5	121.4	121.4	108.18	108.1	d
6	149.3	149.4	166.56	166.18	s
7	124.5	124.4	106.78	106.7	d
8	162.4	162.4	162.52	165.5	s
8’	113.7	113.7	113.69	112.9	s
9	192.5	192.7	190.79	190.69	s
9’	115.9	115.9	113.59	113.55	s
10	181.9	182.0	181.95	181.89	s
10’	133.6	133.7	135.27	133.17	
Ar-CH_3	22.3	22.3	22.02	22.4	q
O-CH_3			56.06 56.06	56.1	q

*Coopoosamy and Magwa, 2006; *[a]Parameswaran et al., 2004.

Table 4. Cytotoxic and antiviral activity of compounds isolated from *Senna siamea.*

Compound	Name	MNTC (µg/mL)	CC_{50} (µg/mL)	IC_{50} (µg/mL)	SI
1	Lupenone	NT	NT	NT	NT
2	Chrysophanol	10.0	14.77	0.46	32. 10
3	Physcion	10.0	38.04	1.57	24.30
4	Lupeol	0.10	0.39	0.014	27.86
5	Betulinic acid	10.0	50.46	21.40	2.35
6	β-Sitosterol-3-*O*-β-D-glucopyranoside	NT	NT	NT	NT

MNTD = maximum non-toxic concentration, CC_{50}= 50% cytotoxic concentration, IC_{50}= 50% inhibitory concentration, SI= selective index, NT = Not tested

psycion and β-sitosterol glucoside.

Lupeol, a triterpenoid, was obtained from both hexane and chloroform soluble fraction of *S. siamea.* Its activity against poliovirus in this study was very significant with IC_{50} 0.14 µg/mL, it was also significantly cytotoxic with CC_{50} 0.39 µg/mL, but the SI which is the ratio CC_{50}/IC_{50} was 27.86 (Table 4), giving the compound a good safety margin despite its toxicity in rhabdomyosarcoma cells. It had been previously shown to possess varying degrees of cytotoxic activities **(**Aratanechemuge et al; 2004; Hata et al., 2003; Saleem et al., 2004). Lupeol had also been shown to possess antiviral activity; it had been reported to inhibit poliovirus, HIV-1 protease, it possess significant antiviral activity against herpes simplex virus (EC_{50} 2.98-4.2 µg/mL) and a host of other viruses (Ryu et al.,1992; Wei et al., 2008).

Chrysophanol and physcion, are 1, 8-dihydroxyanthraquinone derivatives (1, 8-DAD), both were isolated as orange yellow crystals from the hexane soluble fraction of *S. siamea*, they belong to a family of naturally occurring anthraquinones (Dave and Ledwani, 2012). They inhibit poliovirus-induced cytopathic effect in rhabdomyosarcoma cell with IC_{50} values of 0.4571 and 1.568 µg/mL, respectively (Table 4). In earlier study by Semple and co-workers (2001) on poliovirus types 2 and 3, chrysophanol inhibited poliovirus-induced cytopathic effects in BGM kidney cells with EC_{50} of 0.21 and 0.02 mg/mL, respectively. This study established the inhibitory activity of chrysophanol on poliovirus type 1 with IC_{50} of 0.4571 µg/mL. Chrysophanol has been shown also, to have strong inhibitory effect against hepatitis B virus (Li et al., 2004). The presence or absence of a methoxy

group in phenolics could determine the degree of their antimicrobial activity and may be responsible for the differences in the antipoliovirus activity (Moo-Puc et al., 2007) of the more active chrysophanol, as compared to physcion, displayed by their respective inhibition of poliovirus-induced cytopathic effect in rhabdomyosarcoma cell with IC_{50} values of 0.4571 and 1.568 µg/mL, respectively.

The 1, 8-dihydroxyanthraquinone derivatives or crude extracts containing them have been shown severally to possess antimicrobial activity. This activity has been reported to be due to their inhibitory activity on the enzymes which are necessary for the survival of the particular microorganism (Abu-Darwish and Ateyyat, 2008)

The pentacyclic triterpenoid, betulinic acid, has long been recognized as an anticancer agent, it was the least toxic compound of all tested with CC_{50} of 50.46 µg/mL (Table 4), weak anti-poliovirus activity of 21.40 µg/mL and low SI. It was earlier identified as a highly selective growth inhibitor of human melanoma, neuroectodermal and malignant tumor cells (Schmidt et al., 1997). betulinic acid has been shown to inhibit HIV-1 replication and HIV-1 entry (Mayaux et al., 1994), HIV-protease and reverse transcriptase (RT) (Xu et al., 1996). It was reported to enter phase 2 clinic trials as a new AIDS drug candidate (Beatty et al., 2005).

Conclusions

One of the greatest significance of plant derived antiviral metabolites is that they provide prototypes or templates that can be used in the design of potentially superior new chemotherapeutic drugs. It has been stated that for a drug candidate to be considered as an anti-poliovirus drug, it must be able to inhibit the three strains of the virus (polioviruses 1, 2 and 3); chrysophanol is therefore a suitable drug candidate meeting this criterion, as it has earlier been shown by Semple and co-workers, that chrysophanol isolated from *Daniella longifolia* inhibits poliovirus 2 and 3 (Semple et al., 2001). This study has established its inhibitory properties against poliovirus 1 *in vitro*.

Part of the NRC (2006) recommendation was that an ideal poliovirus drug must be one that is also active on other enteroviruses; we will consider screening chrysophanol on most commonly encountered enteroviruses and its activity will also be established on vaccine derived poliovirues (VDPV) especially a circulating vaccine-derived poliovirus (cVDPV). It is worthy of note that all the isolated compounds, except betulinic acid have good selective indices

Conflict of Interests

The authors have not declared any conflict of interests.

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