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Anti-Trypanosomal Activity and Cytotoxicity of Some Compounds and Extracts from Nigerian Medicinal Plants

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1. Introduction

Human African trypanosomiasis, also known as sleeping sickness, is a vector-borne parasitic disease. The parasites concerned are protozoa belonging to the *Trypanosoma* genus. They are transmitted to humans by tsetse fly (*Glossina* genus) which have acquired their infection from human beings or from animals harbouring the human pathogenic parasites. Rural populations living in regions where transmission occurs and which depend on agriculture, fishing, animal husbandry or hunting are the most exposed to the disease. Sleeping sickness threatens millions of people in 36 countries in sub-Saharan Africa. Many of the affected populations live in remote areas with limited access to adequate health services. Treatment depends on drugs that can cross the blood-brain barrier to reach the parasite. Such drugs are toxic and complicated to administer. Suramin, discovered in 1921, is one of the drugs used for the treatment. However, it provokes certain undesirable effects, in the urinary tract and allergic reactions. Other drugs in use are equally toxic and difficult to administer. Thus the need to develop new drugs is imperative. Natural products and medicinal plants (Antia et al, 2009; Sara et al, 2004; Bizimana et al., 2006; Okpekon et al., 2004) have continued to contribute to the search for new and potent anti-trypanosomal drugs with minimal side effects. Their continued relevance in drug discovery and development is due to their bioactivity, biodiversity and reported safety in their use. An analysis of new and approved drugs for the treatment of human diseases indicated that natural products have continued to play a highly significant role in the drug discovery and development process. Thus biologically active natural products from plants, their derivatives or analogues contributed up to 57% of top selling prescription drugs in the United States in 1997 (Newman et al., 1997). This dominant role is due to the leads for the development of drugs that natural products give. Thus expanding, not decreasing, the exploration of nature as a source of novel active agents that may serve as leads and scaffolds for elaboration into desperately needed efficacious drugs for a multitude of disease indications is advocated (Newman & Cragg, 2007). Ethnobotanical surveys have revealed the use of *Alcornea cordifolia*, *Euphorbia poisonii*, *Monodora myristica*, *Prosopis africana*, *Spondias mombim, Nauclea pobeguinii, Terminalia avicennioides, Cochlospermum planchonii, Nauclea latifolia* and *Withania somnifera* in the traditional medicine in parts of Nigeria (Igoli et. al., 2005). These plants are used as spices and medicinally for the treatment of diarrhoea,

dysentery, diabetes, bacterial infections, fevers, pain and snake bites amongst other ailments by the Igede people of Benue State in Nigeria. *Maytenus laevis* is used in traditional medicine in Colombia (Nagakawa et.al., 2004; Gonzalez et al., 1982) against pain and arthritis. Before the introduction of synthetically prepared medicines, herbal remedies were commonly prescribed and were often effective. Thus herbs and spices played very important, sometimes magical, roles in medicine. Hence these plants were collected and their extracts/ compounds isolated were evaluated for anti-trypanosomal and cytotoxic activities. A high throughput (HTS) method was used to screen the extracts and compounds. This has become very useful in modern drug discovery from natural products. It is a form of standardised and replicate screening method which employs 96 well plates which can be used to screen 80 extracts, compounds or fractions at the same time. It can also be adapted to obtain data to plot dose response curves and determine IC_{50} and MIC values. Using a multilabel counter, suitable indicators, wells with extracts, cells, organisms and controls can be read quickly by examining fluorescence, luminescence and optical density or by radiometry giving exact and replicable values free from errors of visual determinations or measurements. The volumes used in these wells are between 200-250µL and for half well plates 20-40µL such that solutions of extracts or compounds as low as 1mg dissolved in 100µL gives a stock solution of 10mg/ml which can be diluted for all range of concentrations typically from 20-30µg/ml to ng or µM to nM values using serial dilution methods. This greatly enhances speed and efficacy of screening for hit molecules (Gray et al., 2011). Efforts were made to identify the active compounds contained in the extracts as much as possible. This is because it is the practice today to screen more and more pure compounds rather than crude extracts or fractions. Screening of crude extracts or mixtures/fractions does not allow understanding of the mode of activity of the test compounds against the target cells or tissues. Isolation of the active compounds is required before active concentrations can be determined. We hereby report on these plant extracts, the compounds obtained from them, their *in vitro* activity against the bloodstream form of *Trypanosoma brucei brucei* (*T.b.brucei*) and the cytotoxic activity of the active compounds/extracts against PNT2A cells.

2. Materials and methods

Standard methods of extraction and isolation or purification of compounds/extracts from plant materials were used while a standardised 96 well based assay was used to screen the extracts for anti-trypanosomal activity and cytotoxicity. The crude extracts as well as pure compounds obtained were screened *in vitro* for trypanosomal activity against T*rypanosoma brucei brucei* (S427) blood stream forms. Active compounds from the preliminary screens were further tested to confirm activity and determine their minimum inhibitory concentration (MIC) values. Cytotoxicity of the active compounds and extracts was then tested against PNT2A cells ("normal" prostatic cells).

2.1 General bioassay

The anti-trypanosomal and cytotoxicity assays were performed using the REDOX indicator Alamar blue™. The active component of which is resazurin (blue in colour) which in the presence of live parasites or cells is reduced to the bright pink fluorescent resorufin. The fluorescence values for the test plates are measured using a microplate reader in fluorescence mode with excitation and emission wavelengths of 560 and 590nm respectively.

Wells containing active compounds are easily identified as they remain blue in colour and have background levels of fluorescence. The test samples are initially screened at a single concentration and then MIC or IC_{50} values are determined for the active compounds at n=2 or n=3. Positive and negative controls and a sterility checks are included in all assays. The incubation and treatment times, incubation conditions and seeding densities are optimised for each test species and cell line.

2.1.1 Anti-trypanosomal activity

Samples were tested against the bloodstream form of *Trypanosoma brucei brucei* (*T.b.brucei*) S427. The activity of the plant extracts and isolated compounds was determined using the well-established Alamar blue™ 96 well microplate assay. Samples were initially screened at a single concentration usually 20µg/ml for extracts or 20µM for pure compounds. The concentration of dimethylsulphoxide (DMSO) should not exceed 0.5% in the initial screen.

Samples for testing were prepared as 10mg/ml or 10mM stock solutions in 100% DMSO. These were diluted with HMI-9 medium to a concentration of 1mg/ml /1mM. 4µl of the test sample was added to the assay well then 96µl HMI-9 medium to give a final assay concentration after a 1:1 dilution of 20µg/ml / 20µM.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	Sterility control	1	2	3	4	5	6	7	8	9	10	1.0µM suramin
B	control	11	12	13	14	15	16	17	18	19	20	0.5µM suramin
C	control	21	22	23	24	25	26	27	28	29	30	0.25µM suramin
D	control	31	32	33	34	35	36	37	38	39	40	0.125µM suramin
E	DMSO control	41	42	43	44	45	46	47	48	49	50	0.062µM suramin
F	DMSO control	51	52	53	54	55	56	57	58	59	60	0.031µM suramin
G	DMSO control	61	62	63	64	65	66	67	68	69	70	0.015µM suramin
H	DMSO control	71	72	73	74	75	76	77	78	79	80	0.008µM suramin

Table 1. Anti-trypanosomiasis assay plate layout.

The screening plate was arranged as illustrated in Table 1. Controls including a sterility control and DMSO controls in column 1; test samples in columns 2 to 11 and a concentration range of suramin as a positive control in column12. Trypanosomes were counted using a haemocytometer and diluted to a concentration of $3x10^4$ trypanosomes /ml, 100µl of this suspension was added to each well of the assay plate with the exception of well A1 the

sterility check. The assay plate was incubated at 37ºC, 5% CO_2 with a humidified atmosphere for 48 hours. After which 20µl of Alamar blue was added and the incubation continued for a further 24 hours. Fluorescence was then determined using the Wallac Victor microplate reader (Excitation 530nm Emission 590nm). The results were calculated as % of the DMSO control values. Minimum inhibitory concentration values (MICs) were determined for samples with less than 10% of control values. MIC determinations were carried out in duplicate. 200µg/ml test solutions were prepared in column 2 by pipetting 4µl of (10mg/ml) test stock solution and 196µl HMI-9 medium into each well. 100µl HMI-9 medium was pipetted into columns 1 and 3-12 and 1:1 serial dilutions were carried out from columns 2 to 11. 80µl of HMI-9 medium was added to column 12 and 20µl of x10 concentrations of suramin to give a final concentration range of 0.008 to 1.0µM. An inoculum of 100µl of trypanosomes at a concentration of $3x10^4$/ml was added to each well except A1, and the procedure continued as previously described.

	1	2	3	4	5	6	7	8	9	10	11	12
A	control	100	50	25	12.5	6.25	3.1	1.55	0.78	0.34	0.17	1.0µM suramin
B	control	100	50	25	12.5	6.25	3.1	1.55	0.78	0.34	0.17	0.5µM suramin
C	control	100	50	25	12.5	6.25	3.1	1.55	0.78	0.34	0.17	0.25µM suramin
D	control	100	50	25	12.5	6.25	3.1	1.55	0.78	0.34	0.17	0.125µM suramin
E	control	100	50	25	12.5	6.25	3.1	1.55	0.78	0.34	0.17	0.062µM suramin
F	control	100	50	25	12.5	6.25	3.1	1.55	0.78	0.34	0.17	0.031µM suramin
G	control	1% DMSO	0.5% DMSO	0.25% DMSO	0.12% DMSO	0.06% DMSO	0.03% DMSO	0.01% DMSO	0.00% DMSO	0.00% DMSO	0.00% DMSO	0.015µM suramin
H	control	1% DMSO	0.5% DMSO	0.25% DMSO	0.12% DMSO	0.06% DMSO	0.03% DMSO	0.01% DMSO	0.00% DMSO	0.00% DMSO	0.00% DMSO	0.008µM suramin

Table 2. Final assay concentrations µg/ml

MIC values were determined as the concentration calculated to have < 5% of control values.

2.1.2 Cytotoxicity determinations

Cytotoxicity determinations were carried out using a modification the Alamar blue™ redox-based microplate assay (O'Brien *et al.*, 2000). The cytotoxic activity of the fractions and compounds was initially determined against PNT2A cells a "human" normal prostatic epithelial cell line. Initial screening was carried out at a concentration of 100µg/ml. Concentration response studies (300 to 0.3µg/ml) were then carried out in duplicate for samples with less than 60% of control values in the initial screen.

Cells were seeded into 96 well microplates at a density of 0.5 x 10^4 cells/well in 100µl Dulbecco's modified Eagle's medium (DMEM; Invitrogen) and incubated at 37°C, 5% CO_2 with a humidified atmosphere for 24 hours. After which DMEM solutions of test compounds at the desired concentrations and DMSO as a negative control and 0.1% Triton X as a positive control were added to give a total well volume of 200µl. The microplates were incubated for 24 hours before the addition of 10µl of alamar blue. After a further 20 hours incubation fluorescence was determined using a Wallac Victor microplate reader in fluorescence mode (Excitation 530nm; Emission 590nm). The results were calculated as % of the DMSO control values. IC_{50} values were calculated for the concentration response data using Graphpad prism software.

2.2 Isolation and structure elucidation of compounds

Melting points (uncorr.) were determined on a Buchi B-540 melting point apparatus. The ^1H NMR and ^{13}C NMR spectra were run on a Bruker AV 300 (400MHz) and DRX 500 spectrometers using $CDCl_3$ or DMSO-d6 as solvents and TMS as internal standard. HRESI-MS were run using Thermo LTQ Orbitrap or Thermo Exactive orbitrap mass spectrometer. A capillary voltage of 46.00V for the positive mode and -48.00V for the negative mode was used for HRESI-MS. Column chromatography were performed using silica gel MN-60 (Macherey-Nagel GmbH & Co. KG) and Gel filtration chromatography using Sephadex LH-20 (GE Health Lifescience UK) were performed in glass columns. Spots on TLC were visualised using Anisaldehyde-H_2SO_4 reagent or Dragendorff's reagent for the alkaloids. Developed TLC plates were also observed under UV lamp using short (λ= 254 nm) and long (λ= 366 nm) wavelengths. The structures of the pure compounds were determined using spectroscopic methods (1D/2D NMR and mass spectrometry). Column chromatography (CC) were carried out on wet packed silica gel in glass columns eluting gradient wise with hexane, hexane-ethyl acetate, ethyl acetate and ethyl acetate-methanol mixtures or with Sephadex LH-20 pre-soaked and wet packed in methanol. After the introduction of the extracts or fractions in methanol, the Sephadex columns were also eluted with methanol. TLC analysis was performed on pre-coated silica gel aluminum plates cut to desired size. NMR experiments were run in $CDCl_3$ or DMSO-d6 with TMS as internal standard on Bruker AV 300 (400MHz) or DRX-500 spectrophotometer.

2.2.1 Plant materials

All plant materials were collected from Oju LGA Benue State, Nigeria between July and September 2008. The plants were authenticated by the Department of Forestry and Wildlife of the University where voucher specimens were deposited in the University of Agriculture Forest Herbarium. *Alcornea cordifolia (*bark), *Euphorbia poisonii* (stem), *Monodora myristica* (seeds), *Prosopis Africana* (bark), *Spondias mombim* (root), *Nauclea pobeguinii* (bark), *Nauclea latifolia* (bark), *Cochlospermum planchonii*(root) and *Withania somnifera* (whole plant), *Terminalia avicennioides* (bark). *Maytenus laevis* (root) was from samples already housed at the Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS). They were all sun dried in open air and ground using a mill.

2.2.2 Extraction and identification of the components

The plant materials (500g – 1.0kg) were air dried and extracted using hexane, ethyl acetate and methanol in a Soxhlet apparatus. The crude extracts were allowed to stand and sometimes cooled to allow for the precipitation of compounds. Where all precipitates have been produced or no (further) precipitation, the crude extracts or precipitates (where not a pure compound) were subjected to column chromatography (CC) eluting gradient- wise using hexane-ethyl acetate and subsequently ethyl acetate-methanol solvent mixtures. Using TLC as a guide, similar fractions were combined and allowed to evaporate under the fume hood to allow for the crystallisation of pure compounds. Where no pure compounds were separated and the fractions were active, such fractions were further purified via preparative thin layer chromatography or Gel filtration (GF) using Sephadex LH-20 to obtain the pure compounds. Methanol extracts which were usually viscous and complex were subjected to an initial vacuum liquid chromatography (VLC) to fractionate the extracts. Similar fractions (TLC) were also combined and further purified via column chromatography or Gel filtration. Flash chromatography using an iES Reveleris Automated Flash Chromatography system was used for the isolation of certain compounds. The column was dry packed after taking up the extracts with silica gel and then loaded onto the pre-packed flash column. The compounds were eluted starting with hexane with incremental additions of ethyl acetate, then with ethyl acetate with additions of methanol and finally with methanol. Using two variable length UV detectors and one ELSD detector, similar eluates were combined and confirmed by TLC and ^{1}H NMR. Pure compounds were then allowed to settle out from the combined fractions. All columns were exhaustively eluted and washed with methanol. Washings were allowed to stand to allow for the precipitation of compounds. The identification of pure compounds was carried out using one dimensional ^{1}H and ^{13}C NMR spectroscopy (including *J*-mod, DEPT-q and DEPT 135 or 90). Spectral data of known compounds were compared with published spectral data and were thereby identified. Further 2D experiments (including correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC) and nuclear overhauser enhancement spectroscopy (NOESY)) were carried out for more complex molecules for accurate assignments of proton and carbon chemical shifts. Assigned structures were confirmed using ESI-MS and HRESI-MS to confirm their mass and molecular formula. The various extracts produced are listed in table 3 below. Extract code descriptions or extensions are: precipitate (P), latex (L), Sephadex (S), methanol wash (w), VLC fraction (V), flash chromatography fraction (F), root (R), bark (B).

3. Results

3.1 Anti-trypanosomal screening

The photographs below show the results from the concentration response studies to determine the MIC of the triterpenoids (compounds 11 and 12) from *M.laevis.* Well A1 is the sterility check, wells B1 to H1 negative controls and wells G2 to G11 and H2 to H11 are the DMSO 1:1 dilution controls. Column 12 contains a concentration range of 1 to 0.008µM of the positive control suramin. In plate 1 compounds 11 (rows C and D) and 12 (rows E and F) are tested n=2 over a concentration range of 100 to 0.17µM. In plate 2 compounds 11 (rows A, B and C) and 12 (rows D,E and F) are tested n=3 over a concentration range of 10 to 0.02µM.

S/No	Plant	Part	Extract codes			
			Hexane (H)	Ethyl acetate (E)	Combined hexane + ethyl acetate (HE)	Methanol (M)
1	*Alcornea cordifolia*	Bark (B)	ACBH	ACBE	ACBHE	ACBM
2	*Cochlospermum planchonii*	Root (R)	CPH	CPE	CPHE	CPM
3	*Euphorbia poisonii*	Stem	EPH	EPE	EPHE	EPM
4	*Maytenus laevis*	Root (R)	MLH	MLE	MLHE	MLM
5	*Monodora myristica*	Seed	MMH	MME	MMHE	MMM
6	*Nauclea latifolia*	Bark (B)	NLH	NLE	NLHE	NLM
7	*Nauclea pobeguinii*	Bark (B)	NPH	NPE	NPHE	NPM
8	*Prosopis Africana*	Bark (B)	PAH	PAE	PAHE	PAM
9	*Spondias mombim*	Root (R)	SMH	SME	SMHE	SMM
10	*Terminalia avicennioides*	Bark (B)	TAH	TAE	TAHE	TAM
11	*Withania somnifera*	Whole plant	WSH	WSE	WSHE	WSM

Table 3. Extracts produced from the plant materials

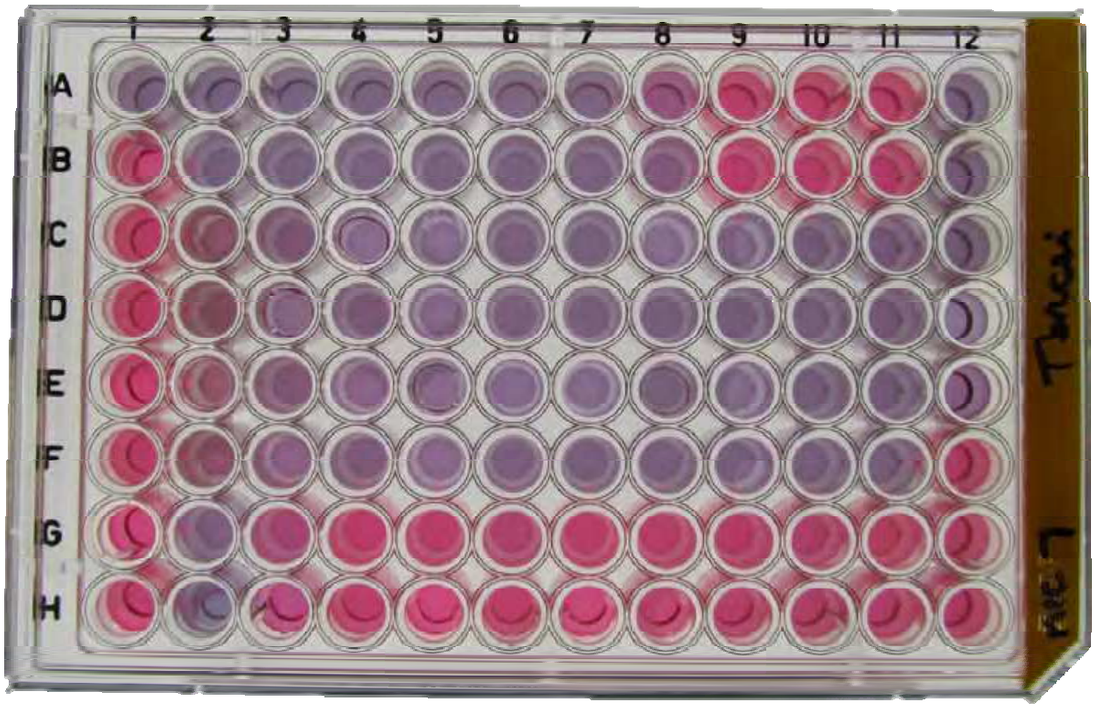

Plate 1. Photo showing MIC of compounds **11** and **12**.

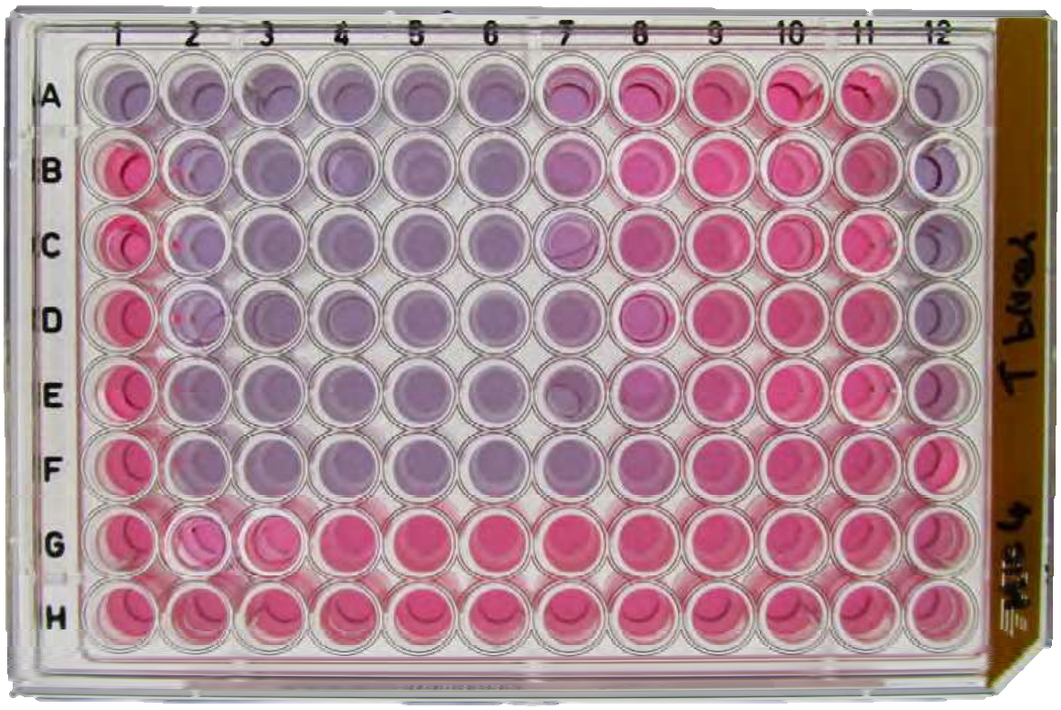

Plate 2. Photo showing MIC of compounds **11** and **12**.

The anti-trypanosomal activity and cytotoxicity against PNT2A cell lines observed for the extracts, fractions and compounds are given in the table below.

	Extract/ Fraction	Compound/ Remarks	Anti-trypanosomal activity		Cytotoxicity	
			% of Control (20 µg/ml)	MIC (µg/ml)	% of Control (100 µg/ml)	IC_{50} (µg/ml)
1	ACBE-1	**8**	13.3		42.8	
2	ACBM-12	**3**	3.8	<0.2	29.9	1.5
3	ACBM-14	**7**	18.0		28.5	24.2
4	PAM-5	Column fraction	12.5			
5	PAM-6	Column fraction	19.4			
6	WSE-1a	Column fraction	12.5	12.5		
7	WSE-1b	Column fraction	12.5	12.5		
8	WSE-1c	Column fraction	3.12	3.12		
9	MMH-1	Crude extract	8.0	12.5		
10	MME-5	Column fraction	-1.2	3.125		
11	MME-10	Column fraction	-1.4	3.125		
12	MMS-28	**9** & **10**	-1.4	6.25		
13	MME-29	Column fraction	-1.3	3.125		
14	MME-41	**10**	6.5	12.5		
15	MME-42	**9**	6.5	25		
16	EPLE-1	Column fraction	0.8		64.5	

	Extract/ Fraction	Compound/ Remarks	Anti-trypanosomal activity		Cytotoxicity	
			% of Control (20 µg/ml)	MIC (µg/ml)	% of Control (100 µg/ml)	IC_{50} (µg/ml)
17	EPLE-5	Column fraction	-0.1	6.25	29.6	
18	EPP-1	**6**	1.3	1.56		
19	EPH-1	Crude extract	-0.2			
20	EPHE-w	**5**	-0.7			
21	TASW-2	**14**	33.6			
22	TAHE-1	Crude extract	8.7			
23	TAHE-4	Column fraction	5.2	2.5		
24	NPE-5	Column fraction	0.8			
25	NPE-7	Column fraction	4.9	6.25	9.7	>100
26	NPE-13	Column fraction	4.4	12.5	44.2	>100
27	NPE-48	**1**	6.4	12.5	42.8	>100
28	NPM-10	Column fraction	28.1		43.9	7.7
29	SMHE-44	**2**	-0.2	25	87.5	
30	SMM-9	**4**	31.6		50	
31	MLHEF-14	**11**	3.8	0.625		
32	MLHEF-18	**12**	6.1	0.625		
33	WSM-w	**13**	78.7			
34	WSH-1	Crude extract	50.0	50.0		
35	WSH-2	Column fraction	25.0	25.0		
36	WSH-3	Column fraction	50.0	50.0		
37	MMH-3	Column fraction	6.25	6.25		
38	MMH-0	Crude extract	25.0	25.0		
39	MMH-4	Column fraction	50.0	50.0		
40	ACRHE-20	Column fraction	20		85.0	
41	NPE-57	**15**	100		87.8	
42	NPE-81	**16**	100			
43	EPH-1.4	Column fraction	100		81.2	
44	EPH-1.1	Column fraction	-0.2	12.5	76.4	
45	EPH-1.6	Column fraction	107.8		79	
46	ACBME-2	Column fraction	91.0		83.2	
47	ACBHE-6	Column fraction	80.3		89.3	
48	SMHE-7	Column fraction	92.3		92.7	
49	SMHE-13	Column fraction	96.3		87.5	
50	ACRM-1	Crude extract	95.8		87.5	
51	MMM-1	Crude extract	108.2			
52	MMEV-12	VLC fraction	-0.4			
53	MMEV-17	VLC fraction	0.9			

Table 4. Activity of crude extracts, fractions and compounds

3.2 Isolated compounds

The following compounds were isolated from the plants:

Compound	Reference	Plant
1	Hotellier et al. (1980)	*Nauclea pobeguinii, Nauclea latifolia*
2	Nonaka et al. (1982)	*Spondia mombin*
3	Lee et al. (1992)	*Alcornea cordifolia*
4	Nagle et al. (2006)	*Cocholospermum planchonii, Spondias mombin*
5	Gewali et al. (1990)	*Euphorbia poisonii*
6	Dubery et al. (1999)	*Euphorbia poisonii*
7	Bankova (1990); Saha et al. (1991)	*Alcornea cordifolia*
8	Koetter et al. (1989)	*Alcornea cordifolia*
9	Nkunya et al. (2004)	*Monodora myristica*
10	Ishii et al (1975)	*Monodora myristica*
11	Gonzalez et al. (1982)	*Maytenus laevis*
12	Jeller et al. (2004)	*Maytenus laevis*
13	Nandy et al. (1989)	*Withania somnifera*
14	Kim et al. (2001)	*Terminalia avecinnoides*
15	Cerri et al.(1988)	*Nauclea pobeguinii, Nauclea latifolia*
16	Cerri et al. (1988)	*Nauclea pobeguinii, Nauclea latifolia*

Table 5. Isolated compounds and plant sources

Some of the active compounds were identified (Mass spec and NMR: 1D, 2D including COSY, HSQC, HMBC, NOESY) their spectral data were compared with literature reports. The structures of the compounds are given in the figure below.

3.3 NMR data for some isolated compounds

Position	Compound 9		Compound 10	
	^{1}H	^{13}C	^{1}H	^{13}C
1	8.57 *br.s* (NH)	-	8.36 *br.s* (NH)	-
2	7.34 *dd* (*J*=2.8, 3.2 Hz)	126.0 (CH)	7.27 *m*	125.4 (CH)
3	6.74 *ddd* (*J*= 0.8, 2.0, 3.2 Hz)	104.5 (CH)	6.62 *m*	103.5 (CH)
4	8.20 *d* (*J*= 1.2 Hz)	126.2 (CH)	7.85 *d* (*J* = 0.8 Hz)	122.7 (CH)
5	-	129.8 (C)	-	126.5 (C)
6	7.80 *dd* (*J*= 1.2, 8.4 Hz)	122.4 (CH)	7.48 *dd* (*J* = 8.4, 1.6 Hz)	121.8 (CH)
7	7.50 *d* (*J*= 8.4 Hz)	111.7 (CH)	7.43 *d* (*J* = 8.4 Hz)	111.6 (CH)
8	-	138.8 (C)	-	137.9 (C)
9	-	127.8 (C)	-	128.2 (C)
1`	10.06 *s*	192.5 (CHO)	2.41 *s*	27.4 (CH_3)
2`	-	-	-	198.6 (CO)
3`	-	-	6.73 *d* (*J* = 16.4 Hz)	124.7 (CH)
4`	-	-	7.69 *d* (*J* = 16 Hz)	145.6 (CH)

Table 6. The NMR spectra assignments for compounds **9** and **10**.

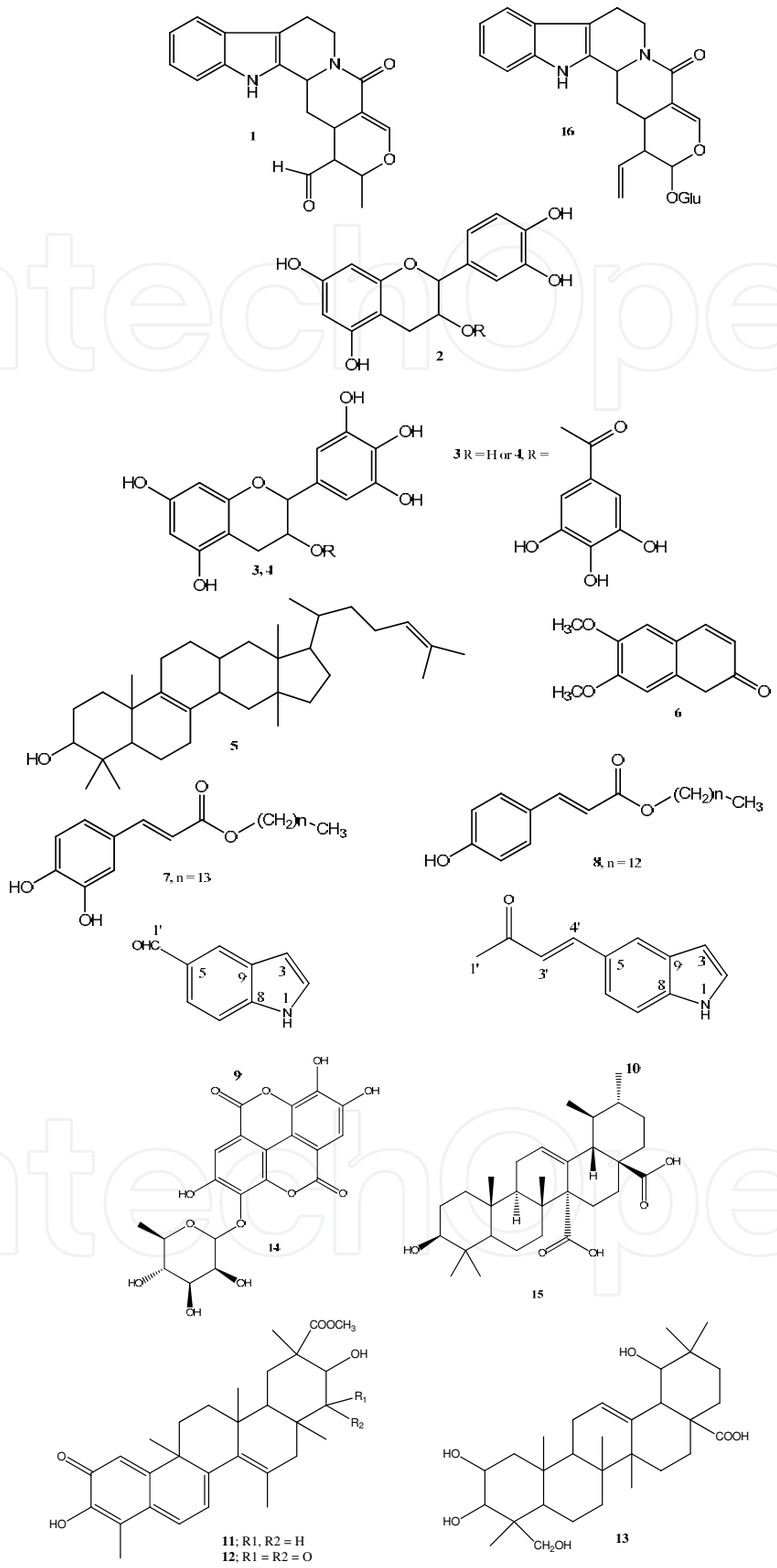

Fig. 1. Structures of isolated compounds

4. Discussion

The beta carboline alkaloids were isolated from the two *Nauclea spp.* The cathechins and their gallates were obtained from *A. cordifolia, S. mombim and C. planchonii*. Catechins including catechin, epicatechin, gallocatechin and five others have been reported to be active against nonproliferative bloodstream trypomastigote and intracellular replicative amastigote parasite forms of *Trypanosoma cruzi* (Paveto et al., 2004). The stem of *E. poisonii* afforded the steroidal triterpene and scoporone. The caffeic acid esters were isolated from *A.cordifolia* and there could be a mixture of more esters. The quinonemethide friedelene triterpenes were obtained from hexane and ethyl acetate extracts of *M. laevis*. They showed very good activity with MICs of 0.625µg/ml. Other pure compounds obtained are as listed on table 2. Some of the pure compounds were however not active when tested. Compound **13** is one of such as well as compound **14** which did not show very good activity. The results of these screening supports a further investigation of the active compounds identified. These could be confirmed as hit molecules especially when the compound's cytotoxicity is low showing selectivity which is vital for drug molecules. Compounds that are inimical to the growth and survival of microorganisms but do not affect the cells adversely are potential candidates for drug development. Some of the extracts and fractions show significant activity but the active compounds have not been identified in this study. Such extracts could be subject of further investigation to isolate or identify the active components. These results also lend credence to some of the ethnobotanical or traditional use of the plants as they are mainly used against fevers caused by malarial parasites. Interestingly, the same or similar compounds were isolated from the different plant samples. Some belong to the same families but others do not. This thus confirms their activity not being due to the crude extracts or fractions but the fact that these extracts or fractions contain the same or similar compounds. These results may easily pave way for a structure activity study as variations in structure and side chains or substituents on similar moieties vis-à-vis activity can easily be observed. For instance it can be observed that glycosidation knocks off activity from the test compounds.

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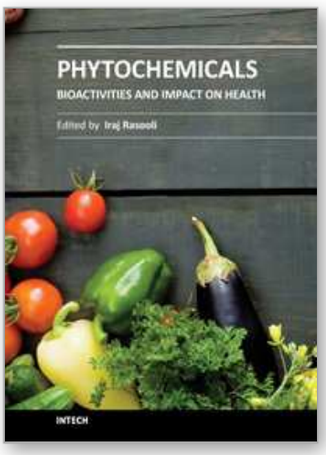

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Among the thousands of naturally occurring constituents so far identified in plants and exhibiting a long history of safe use, there are none that pose - or reasonably might be expected to pose - a significant risk to human health at current low levels of intake when used as flavoring substances. Due to their natural origin, environmental and genetic factors will influence the chemical composition of the plant essential oils. Factors such as species and subspecies, geographical location, harvest time, plant part used and method of isolation all affect chemical composition of the crude material separated from the plant. The screening of plant extracts and natural products for antioxidative and antimicrobial activity has revealed the potential of higher plants as a source of new agents, to serve the processing of natural products.

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