OPEN ACCESS Pharmacologia

ISSN 2044-4648
DOI: 10.5567/pharmacologia.2016.93.102

Research Article
Evaluation of the Sensitivity of Measles Virus and Herpes Simplex Virus-1 to Three Locally Available Herbs in Nigeria

[1,4]R.K. Obi, [2,4]A.A. Anjorin, [3,4]B.O. Oke, [4]O.B. Salu, [5]S.A. Adesegun, [5]T.I. Adeleke, [6]O.A. Olayinka, [6]O.S. Ogunsemowo, [6]J.A. Shenge, [7]O.A. Adedeji, [7]B.O. Ajuwon, [6]J.O. Adeleke, [8]K.M. Salawu and [4]S.A. Omilabu

[1]Department of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria
[2]Department of Microbiology, Lagos State University, Ojo, Lagos State, Nigeria
[3]Clinical Sciences Division, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria
[4]Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Lagos State, Nigeria
[5]Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Lagos State, Nigeria
[6]Department of Virology, College of Medicine, University of Ibadan, Oyo State, Nigeria
[7]WHO Polio Laboratory, Department of Virology, College of Medicine, University of Ibadan, Oyo State, Nigeria
[8]Department of Pharmacognosy and Drug Development, University of Ilorin, Kwara State, Nigeria

Abstract

Background and Objective: Viruses cause a variety of illnesses in humans, yet only a few antiviral drugs have been developed. This study was therefore designed to evaluate three potentially useful medicinal plants locally available in Nigeria for possible therapeutic applications against genetically and functionally diverse virus families public health importance. **Materials and Methods:** Fresh leaves of *Bambusa vulgaris*, *Moringa oleifera* and seed of *Citrus paridisi* were collected from Lagos State, South Western Nigeria. Extraction of the plant materials was done with methanol, chloroform, water and n-hexane using the Soxhlet extractor and concentrated using the rotary evaporator. **Results:** Results obtained showed that n-hexane extracts of *B. vulgaris* and *M. oleifera* inhibited measles virus at the concentrations of 0.125 and 0.016 μg μL^{-1}, respectively. Similarly, while aqueous extract of *M. oleifera* inhibited measles at 0.125 and 0.063 μg μL^{-1}, aqueous extract of *C. paridisi* inhibited the virus at 0.031 μg μL^{-1}. While none of the extracts of *M. oleifera* and *C. paridisi* produced any inhibitory activity against HSV-1, n-hexane extract of *B. vulgaris* was able to inhibit the virus at 0.125 μg μL^{-1}. Results of the mechanism of action of the extracts on the replicative cycle of the viruses revealed both adsorption/entry and post infection inhibitors of the viruses. Phytochemical analysis of the extracts revealed the presence of terpenes, alkaloids, flavonoid, tannins, combined and free anthraquinones, cardiac glycosides and saponins. **Conclusion:** This study has shown that indigenous medicinal plants are invaluable broad spectrum agents in the development of efficacious antiviral cocktails against RNA and DNA viruses of public health importance in Nigeria.

Key words: Plant extracts, antivirals, measles virus, herpes simplex virus-1, phytochemicals

Received: December 13, 2015 Accepted: February 06, 2016 Published: March 15, 2016

Citation: R.K. Obi, A.A. Anjorin, B.O. Oke, O.B. Salu, S.A. Adesegun, T.I. Adeleke, O.A. Olayinka, O.S. Ogunsemowo, J.A. Shenge, O.A. Adedeji, B.O. Ajuwon, J.O. Adeleke, K.M. Salawu and S.A. Omilabu, 2016. Evaluation of the sensitivity of measles virus and herpes simplex virus-1 to three locally available herbs in Nigeria. Pharmacologia, 7: 93-102.

Corresponding Author: R.K. Obi, Department of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Many plants have been used traditionally to enhance host resistance to viral infections and advances in immunology have uncovered possible novel mechanisms for their action to include effects on T helper-1/T helper-2 (TH-1/TH-2) balance, on expression of heat shock protein and interaction with toll-like receptors[1]. To support the immune system, herbalists have traditionally employed herbs that contain chemicals known as high molecular weight heterogenous polysaccharides. Certain types of these chemicals enhance the body's general immunity, for instance by increasing the total number of lymphocytes and helper T-cells or the activity of natural killer cells or by increasing the number of immune stimulating messenger molecules known as cytokines (e.g., interferon, interleukins). Immune enhancing polysaccharides have been identified in herbs such as siberian ginseng, astragalus, liquorice, bladderwrack and saw palmetto[2].

In addition, antiviral compounds from herbs could interrupt the virus replication cycle at one of the stages of the infectious process. For example, the chemical known as prunellin (a sulphated polysaccharide) from *Prunella vulgaris* could block the receptor used by HIV so that viral attachment is prevented. A different chemical from nettle roots (UDA-an N-acetylglucosamine specific lectin) inhibits the same virus but by preventing the genetic information from the virus fusing with the host cell's genome. Furthermore some compounds work by interfering with the enzymes needed to make copies of virus components. Pokeweed antiviral protein works in this way, as does baicalin (from plantain) and skullcap[2].

With a unique plant heritage estimated at over 30000 high quality plant species, of which over 3000 are used in traditional medicine, Africa is in a favorable position to explore the traditional and medicinal uses of plants to the fullest[3]. In Nigeria alone, around 205 of the total medicinal plant species indigenous to Africa are found in nature in the Northern, Western, Central and Eastern zones of the country[4]. The plants are often collected following information supplied by the local healers in geographical areas where they are found[5]. They are then screened for their pharmacological properties. Due to ethical reasons, the *in vitro* tests are the only approach used in these screenings. Many interesting results have been recorded with the crude extracts, thus, showing the rationale behind plant usage in Africa, but it is not enough considering the large amount of plants which have not been screened for their antiviral activities, biochemical compositions or pharmacological properties[5]. This informed the current study, aimed at exploring and evaluating the antiviral properties of extracts from three medicinal plants locally available in Nigeria namely: *Bambusa vulgaris*, *Citrus paridisi* (seed) and *Moringa oleifera*, with a view to finding a permanent cure against some known viruses of public health importance in the country, including measles and herpes simplex-1 virus.

Measles, caused by measles virus, a member of the genus Morbillivirus and family Paramyxoviridae, is a common infection in children[6]. Measles virus is a single stranded, enveloped virus with a non-segmented, negative-strand RNA genome[7]. The virus is highly contagious and transmission is airborne through respiratory droplet nuclei spread or by direct contact with infected nasal or throat secretions. Measles is an epidemic disease of humans and is not spread by any other animal species[8].

Herpes simplex viruses are very large enveloped double stranded DNA viruses with icosahedral symmetry belonging to the family Herpesviridae and subfamily alphaherpes virus[9]. Herpes simplex virus is known to have two serological subtypes, namely, HSV-1 and HSV-2. Typically, HSV-1 is acquired in childhood and causes orolabial ulcers, while HSV-2 is sexually transmitted and causes ulcers of the anal and genital regions. But this classical trend now varies, because both oral infections with HSV-2 and genital infection with HSV-1 are increasingly documented[10].

In the search for drugs and new cures for human and animal ailments, medicinal plants are definitely the most popular targets. Consequently, all parts of the plant body and in fact, various plant products including their extracts, oils, juices and sap have been used in one form or the other in traditional and conventional medicine[11]. In view of this therefore, different parts of indigenous plants were assembled in this study and investigated their antiviral efficacy against known viruses of public health importance in Nigeria especially measles and herpes simplex-1 viruses.

MATERIALS AND METHODS

Study design/study site: This is an *in vitro* tissue culture study using vero cell lines in MEM medium in 96 well tissue culture plates. Initial plant samples preparation and tissue culture study was done at the Virology Research Laboratory, College of Medicine of the University of Lagos, Idi-Araba. Extraction of the plant materials was done at the Pharmocognosy Department, University of Lagos while antiviral evaluation was done at the Department of Virology, University of Ibadan. The study was carried out between July, 2013 and August, 2015.

Collection of plant samples: Fresh and healthy leaves of *B. vulgaris* and *M. oleifera* and seeds of *C. paridisi* were collected from Lagos State, South Western Nigeria. The plants materials were carefully examined then old, insect-damaged and fungus infected samples were removed. The plant samples were authenticated by a Taxonomist at the Herbarium unit of the Department of Botany, University of Lagos, where Voucher specimens were deposited.

Extraction of plant materials: Each plant part was washed to remove impurities and then spread in an airy room for the water to drain. They were then dried using Kottermann hot air oven for 24 h at 45°C. The samples were afterwards blended to a fine powder using Christy and Norris laboratory milling machine set at 8000 rpm. Each powdered material was weighed using Sauter SM 1000 electronic weighing balance and the respective weights (in grams) were noted and recorded.

Thereafter each powdered material was taken to the Soxhlet extractor (Nahalito) for separate extraction using 99% Analar grade of methanol, n-hexane (Merck KGaA, Germany), chloroform (Merck KGaA, Germany) and distilled water, as described by Wang and Weller[12]. Then using a rotary evaporator (Buchi) the extracts were concentrated in vacuo at 60°C to a final volume of 3 mL. The solid residues obtained after evaporation were dried, weighed and the yields were noted and recorded. They were then preserved at -20°C in an airtight sterile McCartney bottle till further use as recommended by Somchit *et al.*[13] and Patrick-Iwuanyanwu *et al.*[14].

Reconstitution of extract: About 10 mg of each dried/pasty solid plant extracts was weighed using electronic weighing balance and introduced into sterile calibrated centrifuge tubes. They were then reconstituted in 1 mL of dimethyl sulfoxide (DMSO) (Sigma) and shaken vigorously to ensure complete dissolution. When the extracts have completely homogenized with the dissolving solvent, they were then brought to a final volume of 10 mL with the addition of 9 mL of sterile distilled water. They were subsequently filtered, first, with 0.45 µm and then with 0.22 µm membrane syringe filters (Cell Treat USA). They were then aliquoted unto sterile plain bottles, closed tightly and stored as stock under refrigeration at -20°C till further use. Hundred microliters of each extract concentration was thereafter used to evaluate the cellular toxicity of the extracts as well as antiviral assay[15].

Phytochemical screening: The extracts were first reconstituted in methanol extraction solvent and then tested by standard phytochemical methods according to Evans[16], for the presence of alkaloids, cardiac glycosides, combined anthraquinones, flavonoids, free anthraquinones, saponins, tannins and terpenes.

Evaluation of cellular toxicity: The method used was based on cellular morphologic changes[17]. Vero cells were prepared at a density of 8×10^4 cells mL^{-1} in a 10% MEM medium in 75 cm^3 tissue culture flasks (Cell Treat, USA). Hundred microliters of this cell suspension (containing 83,333 cells) was then dispensed into each well of a flat bottomed 96-well tissue culture plate (Cell Treat, USA) and incubated for 24 h at 37°C. The growth medium was aspirated after this period, discarded and replaced with 100 µL of 1% MEM medium. Then a 2 fold serial dilution of the extract was carried out using 1% MEM medium as a diluent. The 96 well plates containing the vero cells were labeled with the different dilution and name of each extract. Then using different pipette tips 100 µL of each extract dilution was introduced into each of the wells in duplicates. The last row of wells containing cells but no extracts was used as negative control while the row containing the aciclovir (drug) and cell was used as positive control. The plates were labeled with time and date and incubated at 36.5°C. Cell viability was monitored on days 3, 5, 7, 11 and 14 for any possible immediate changes in morphology known as the cytopathic effect (CPE) compared with the control wells containing only medium and no extract, using an inverted microscope (Inverskop 40C)[17-19]. Complete (100%) CPE was scored as 4^+, 75% as 3^+, 50% as 2^+, 25% as 1^+ and 0 when there is no CPE.

Isolation of test viruses: Measles virus used in this study was isolated from measles vaccine (Edmonston-Zaghreb strain, Serum Institute, Hadaraba, Pune, India), obtained from Institute of Child Health, University College Hospital (UCH), Ibadan, while the second virus, herpes simplex virus-1 (HSV-1) was isolated from whole blood of a male patient who presented with HSV-1 symptoms at the Central Research Lab, Lagos University Teaching Hospital (LUTH). The isolated virus was confirmed to be HSV-1 using PCR technique. The viruses were titrated and using Reed-Muench method, the tissue culture infective doses of the viruses were calculated to be $10^{-3.7}$ $TCID_{50}$ mL^{-1} for measles virus and $10^{-5.7}$ $TCID_{50}$ mL^{-1} for HSV-1. The respective 100 $TCID_{50}$ for both viruses which was used for the screening was $10^{-1.7}$ and $10^{-3.7}$ $TCID_{50}$ mL^{-1}.

Test for virucidal activity: Vero cells were prepared at a density of 8×10^4 cells mL^{-1} in a 10% MEM medium in 75 cm^3 tissue culture flasks (Cell Treat, USA). Hundred microliter of this cell suspension (containing 83,333 cells) was then dispensed into each well of a flat bottomed 96-well tissue culture plate (Cell Treat, USA) and incubated for 24 h at 37°C. Then (a) 200 µL of 100 $TCID_{50}$ virus titer+200 µL of safe concentrations of each test extract and (b) 200 µL of 100 $TCID_{50}$ virus titer +200 µL of 1% medium as a control were prepared and incubated for Ih at 37°C in 5% CO_2. The 10% medium in the 96 multi well plate was aspirated, discarded and replaced with 100 µL of 1% MEM medium[20].

After the 1h incubation period, 100 µL of (a) Virus+extract mixture was inoculated in triplicate unto the 96-well tissue culture plate seeded with Vero cells. Similarly 100 µL of (b) Virus+1% medium mixture was added in triplicate to the last three wells of each row on the same 96-well tissue culture plate to serve as control. Then two-fold serial dilutions using separate pipette tips for each dilution was made starting from the first row downwards, keeping the last two rows of wells as cell control and extract control. All the mixtures were incubated at 37°C in 5% CO_2[19-20].

On days 3-7 after infection, all wells were examined under the inverted microscope (Inverskop 40C) and scored for the presence of virus-induced syncytia as follows[19,21]. The wells containing virus and extract were also scored and compared with the wells containing virus but no extract[22].

Adsorption inhibition test: About 100 µL of Vero cell line was added to each of 96-well of a microtiter plate and incubated for 24 h at 37°C. The medium was aspirated and discarded afterwards. Then 100 µL of different concentrations of each plant extract was added to each well and incubated at 37°C for 2 h in a 5% CO_2. The extracts were thereafter removed after incubation to prevent any interaction with the viruses when the virus inoculum was added. Then 100 µL of 100 $TCID_{50}$ of each virus dilution in 1% MEM medium was added to the wells. This was incubated days and the presentation of CPE was investigated daily for 7 days using an inverted microscope (Inverskop 40C) and scored[19,21].

Post infection inhibition test: About 100 µL of vero cell line was added to each of 96-well of a microtiter plate and incubated for 24 h at 37°C. The medium was aspirated and discarded afterwards. Then 100 µL of 100 $TCID_{50}$ of each virus dilution in 1% MEM medium was added to the wells. The plate was incubated in a 5% CO_2 incubator for 2 h. Thereafter media and unbound virus were washed off and cells were refreshed with 1% MEM medium containing different extract concentrations and incubated at 37°C in a 5% CO_2 incubator and the presentation of CPE was investigated daily for 7 days using an inverted microscope (Inverskop 40C) and scored according to the method of Hierholzer and Killington[21].

RESULTS

This study shows that measles virus and herpes simplex virus-1 are susceptible to indigenous Nigerian medicinal plants. Table 1 shows the plant extracts used in this study contain various types of phytochemicals.

Screening of plant extracts for antiviral potential must be done using non-cytotoxic concentrations of extract. At the end of the period of incubation, the results of the toxicity evaluation of the extracts were as shown in Table 2.

The MNTC of the extracts was used to evaluate the virucidal activity of the extracts on the test viruses is as shown in Table 3-6.

Table 5 and 6 shows the result of the mechanism of action of the extracts on the test viruses.

The result in Table 7 and 8 shows the Inhibitory Concentration (IC_{50}) and Selectivity Index (SI) of the extracts that inhibited the test viruses.

Table 1: Phytochemical screening of the methanol extract of plant extracts

	Constituents										
					Anthraquinones						
	Alkaloid		Cardiac glycosides		Free anthraq	combined anthraq	Flavonoids				
Extracts	Wagner's	Dragendorff's	Kedde's	Kellear-Killiani's	Bontrager's	Modified bontrager's	Ferric chloride	Lead acetate	Saponins	Tannins	Terpenes
B. vulgaris	+	+	++	+	+	+	++	++	++	++	-
C. paridisi	-	+	-	-	-	-	++	++	++	+	-
M. oleifera	++	+	+	++	++	+	++	++	++	+	+++

+++: Very much abundant, ++: Abundant, +: Present, -: Absent

DISCUSSION

Viral infections are a continuing danger to everyone regardless of age, sex, lifestyle, ethnic background and socioeconomic status. Despite the availability of vaccines for measles virus and antiviral drugs for HSV-1, these viruses have continued to cause suffering and death and impose heavy financial burdens on the society, prompting the search for alternative treatment modalities. This study was carried out to determine the sensitivity of measles virus and herpes simplex virus-1 which still constitute a menace to public health in Nigeria to selected herbs: leaves of *Bambusa vulgaris* and *Moringa oleifera* and seed of *Citrus paridisi*. The toxicity of the extracts was first evaluated on vero cells (Table 2) and the Minimum Non-Toxic Concentration (MNTC) or Inhibitory Concentration (IC_{50}) of each extract was used to screen the sensitivity of the viruses to the plant materials.

Table 2: Level of toxicity (IC_{50}) of extracts (µg µL^{-1}) of the plant extracts on vero cell

	Extract concentration (IC_{50})							
Extract	1	0.5	0.25	0.125	0.063	0.031	0.016	0.008
B. vulgaris								
BVM	4$^+$	4$^+$	4$^+$	3$^+$	3$^+$	0	0	0
BVH	4$^+$	4$^+$	3$^+$	0	0	0	0	0
BVC	4$^+$	4$^+$	3$^+$	3$^+$	2$^+$	0	0	0
BVW	4$^+$	4$^+$	3$^+$	2$^+$	0	0	0	0
C. paridisi								
CPM	4$^+$	3$^+$	2$^+$	1$^+$	0	0	0	0
CPH	4$^+$	4$^+$	4$^+$	3$^+$	2$^+$	1$^+$	0	0
CPC	4$^+$	4$^+$	4$^+$	4$^+$	3$^+$	0	0	0
CPW	4$^+$	4$^+$	3$^+$	2$^+$	1$^+$	0	0	0
M. oleifera								
MOM	4$^+$	3$^+$	2$^+$	1$^+$	0	0	0	0
MOH	4$^+$	4$^+$	3$^+$	3$^+$	2$^+$	1$^+$	0	0
MOC	4$^+$	4$^+$	4$^+$	3$^+$	3$^+$	2$^+$	1$^+$	0
MOW	4$^+$	4$^+$	3$^+$	0	0	0	0	0
ACI positive control	1$^+$	1$^+$	1$^+$	1$^+$	0	0	0	0
Extract/cell control	0	0	0	0	0	0	0	0

4$^+$: Complete (100%) cytopathic effect (CPE), 3$^+$: 75% CPE, 2$^+$: 50% CPE, 1$^+$: 25% CPE, 0: No CPE, BVM: *B. vulgaris* methanol, BVH: *B. vulgaris* n-hexane, BVC: *B. vulgaris* chloroform, BVW: *B. vulgaris* water, CPM: *C. paridisi* methanol, CPH: *C. paridisi* n-hexane, CPC: *C. parisi* chloroform, CPW: *C. paridisi* water, MOM: *M. oleifera* methanol, MOH: *M. oleifera* n-hexane, MOC: *M. oleifera* choloform, MOW: *C. paridisi* water, ACI: Aciclovir

Table 3: Virucidal activity of the plant extracts (µg µL^{-1}) against 100 $TCID_{50}$ of measles virus

	Extract concentration (IC_{50})									
Extract	1	0.5	0.25	0.125	0.063	0.031	0.016	0.008	0.004	0.002
B. vulgaris										
BVM						4$^+$	4$^+$	4$^+$		
BVH				0	4$^+$	4$^+$				
BVC						4$^+$	4$^+$	4$^+$		
BVW					4$^+$	4$^+$	4$^+$			
C. paridisi										
CPM					1$^+$	4$^+$	4$^+$			
CPH							4$^+$	4$^+$	4$^+$	
CPC						4$^+$	4$^+$	4$^+$		
CPW						0	4$^+$	4$^+$		
M. oleifera										
MOM					1$^+$	1$^+$	1$^+$			
MOH				0	2$^+$	3$^+$				
MOC								4$^+$	4$^+$	4$^+$
MOW				0	0	1$^+$				
ACI +ve control					4$^+$	4$^+$	4$^+$			

4$^+$: Complete (100%) cytopathic effect (CPE), 3$^+$: 75% CPE, 2$^+$: 50% CPE, 1$^+$: 25% CPE, 0: No CPE, BVM: *B. vulgaris* methanol, BVH: *B. vulgaris* n-hexane, BVC: *B. vulgaris* chloroform, BVW: *B. vulgaris* water, CPM: *C. paridisi* methanol, CPH: *C. paridisi* n-hexane, CPC: *C. parisi* chloroform, CPW: *C. paridisi* water, MOM: *M. oleifera* methanol, MOH: *M. oleifera* n-hexane, MOC: *M. oleifera* choloform, MOW: *C. paridisi* water, ACI: Aciclovir

Table 4: Virucidal activity of the plant extracts (µg µL^{-1}) against 100 $TCID_{50}$ of HSV-1

	Extract concentration (IC_{50})									
Extract	1	0.5	0.25	0.125	0.063	0.031	0.016	0.008	0.004	0.002
B. vulgaris										
BVM						4$^+$	4$^+$	4$^+$		
BVH				0	4$^+$	4$^+$				
BVC						4$^+$	4$^+$	4$^+$		
BVW					4$^+$	4$^+$	4$^+$			
C. paridisi										
CPM					4$^+$	4$^+$	4$^+$			
CPH							4$^+$	4$^+$	4$^+$	
CPC						4$^+$	4$^+$	4$^+$		
CPW						2$^+$	4$^+$	4$^+$		
M. oleifera										
MOM					3$^+$	3$^+$	3$^+$			
MOH							3$^+$	4$^+$	4$^+$	
MOC								4$^+$	4$^+$	4$^+$
MOW				2$^+$	3$^+$	4$^+$				
ACI +ve control					4$^+$	4$^+$	4$^+$			

4$^+$: Complete (100%) cytopathic effect (CPE), 3$^+$: 75% CPE, 2$^+$: 50% CPE, 1$^+$: 25% CPE, 0: No CPE, BVM: *B. vulgaris* methanol, BVH: *B. vulgaris* n-hexane, BVC: *B. vulgaris* chloroform, BVW: *B. vulgaris* water, CPM: *C. paridisi* methanol, CPH: *C. paridisi* n-hexane, CPC: *C. parisi* chloroform, CPW: *C. paridisi* water, MOM: *M. oleifera* methanol, MOH: *M. oleifera* n-hexane, MOC: *M. oleifera* choloform, MOW: *C. paridisi* water, ACI: Aciclovir

Table 5: Adsorption/entry and post infection inhibitory tests of the extracts on 100 $TCID_{50}$ of measles virus

	Plant extract									
	Adsorption/entry inhibition					Post infection inhibition				
Extract dilution	BVH	CPW	MOW	MOH	ACI	BVH	CPW	MOW	MOH	ACI
1										
0.5										
0.25										
0.125	1$^+$		1$^+$			0		0		
0.063		3$^+$	1$^+$		4$^+$	0		0		4$^+$
0.031	4$^+$	0	1$^+$		4$^+$	0	0	0		4$^+$
0.016		0		1$^+$	4$^+$		0		0	4$^+$
0.008	0		2$^+$				0		0	
0.004					3$^+$				0	

4$^+$: Complete (100%) cytopathic effect (CPE), 3$^+$: 75% CPE, 2$^+$: 50% CPE, 1$^+$: 25% CPE, 0: No CPE, BVH: *Bambusa vulgaris* n-hexane, CPW: *Citrus paridisi* water, MOW: *Moringa oleifera* water, MOH: *Moringa oleifera* n-hexane, ACI: Aciclovir +ve control

Table 6: Adsorption/entry and post infection inhibitory tests of the extracts on 100 $TCID_{50}$ of HSV-1

	Plant extract			
	Adsorption/entry inhibition		Post infection inhibition	
Extract dilution	BVH	ACI	BVH	ACI
1				
0.5				
0.25				
0.125	0		4^+	
0.063	3^+	4^+	4^+	4^+
0.031	4^+	4^+	4^+	4^+
0.016		4^+		4^+
0.008				
0.004				

4^+: Complete (100%) cytopathic effect (CPE), 3^+: 75% CPE, 0: No CPE, BVH: *Bambusa vulgaris* n-hexane, ACI: Aciclovir +ve control

Table 7: 50% Inhibitory Concentration (IC_{50}) and Selectivity Index (SI) of extracts that inhibited measles virus

Plant sample	Vero cell IC_{50} (µg µL^{-1})	Virus replication IC_{50} (µg µL^{-1})	SI
Virucidal activity			
BVH	0.125	0.125	1.00
CPW	0.031	0.031	1.00
MOW	0.125	0.063	2.00
MOH	0.016	0.016	1.00
Adsorption/entery inhibitors			
CPW	0.031	0.008	3.86
Post infection inhibitors			
BVH	0.125	0.031	4.03
CPW	0.031	0.008	3.86
MOW	0.125	0.031	4.03
MOH	0.016	0.004	4.00

BVH: *Bambusa vulgaris* n-hexane, CPW: *Citrus paridisi* water, MOW: *Moringa oleifera* water, MOH: *Moringa oleifera* n-hexane

Table 8: 50% Inhibitory Concentration (IC_{50}) and selectivity index (SI) of extracts that inhibited HSV-1

Plant sample	Vero cell IC_{50} (µg µL^{-1})	Virus replication IC_{50} (µg µL^{-1})	SI
Virucidal activity			
BVH	0.125	0.125	1.00
Adsorption/entery inhibitors	None		
Post infection inhibitors			
BVH	0.125	0.125	1.00

BVH: *Bambusa vulgaris* n-hexane

The virucidal activity as shown in Table 3 clearly revealed that the extracts could actually inactivate the viruses. While n-hexane and water extracts for *B. vulgaris* and *C. paridisi*, respectively inhibited measles at concentrations of 0.125 and 0.031 µg µL^{-1}, respectively, two extracts of *M. oleifera* n-hexane and water inhibited the virus at 0.016 and 0.125 µg µL^{-1}, respectively. Water and n-hexane could therefore be regarded as better extraction solvents compared to methanol and chloroform. The virucidal test of HSV-1 as revealed in Table 4 shows that only n-hexane extract of *B. vulgaris* at 0.125 µg µL^{-1} could inactivate the virus before its introduction into the cell. The result shows that the two viruses which have actually defied orthodox treatment could be susceptible to herbs freely available in Nigeria. For the plants to have inactivated both viruses it means the plants possess the constituents that could overcome the viral envelopes, capsids and other protective devices of both viruses and reach the viral genome which is the single RNA strand of measles[7] and the double DNA strand of HSV-1[23].

The mechanism of action of the extracts on the replicative cycle of the viruses revealed that *Citrus paridisi* could be a prophylactic agent of measles infection (Table 5) having prevented attachment/entry of the virus to the cell, thus acting as an attachment/entry inhibitor of measles virus. The same plant material could also be used for treatment of infection by the virus having inhibited progression of the disease after attachment. Similarly, *B. vulgaris* and *M. oleifera* could be potential agents for treatment of measles infection since they could also exert their activity in the post infection activity of the virus (Table 5). It is possible that the plants which could not allow progression of measles after attachment inhibited one of the steps in the replicative cycle of the virus thereby preventing the virus from completing its infectious process. The only plant that could inhibit HSV-1 was *B. vulgaris* and it could only exert this effect before virus attachment showing that this plant, which could be a potent attachment/entry inhibitor of HSV-1, could be a veritable prophylactic agent against infection by the virus (Table 6). It is possible HSV-1 post infection activity could not be inhibited because of the ability of HSV-1 to establish latency after initial infection[24] which may have been the reason why the plant extract could not reach the virus to inhibit it.

The clinical value of the extracts was determined by their Selectivity Index (SI). All the extracts have SI values which exceeded the IC_{50} of the extracts (Table 7 and 8). As reported by De Clercq,[25] a compound with a low IC_{50} and a high SI is most likely to have value as an antiviral drug. As a result therefore all the extracts which showed inhibitory activities against the viruses in this study could become potential antiviral drugs and could safely be administered for the prevention and treatment of infections caused by the viruses since they all showed SI values above the IC_{50} of the extracts.

The antiviral activities of the plants observed in this study may not have been unconnected with the abundance of phytochemicals present in their tissues (Table 1). Quinones are known to form irreversible complexes with nucleophilic amino acids in proteins[26] often leading to inactivation of the protein and loss of function, a property responsible for the great potential antimicrobial effects of this phytochemical[27]. Flavonoids or biflavonoids have been shown to have antiviral activities on certain viruses: morin (anti-HSV-2 activity), iridoid

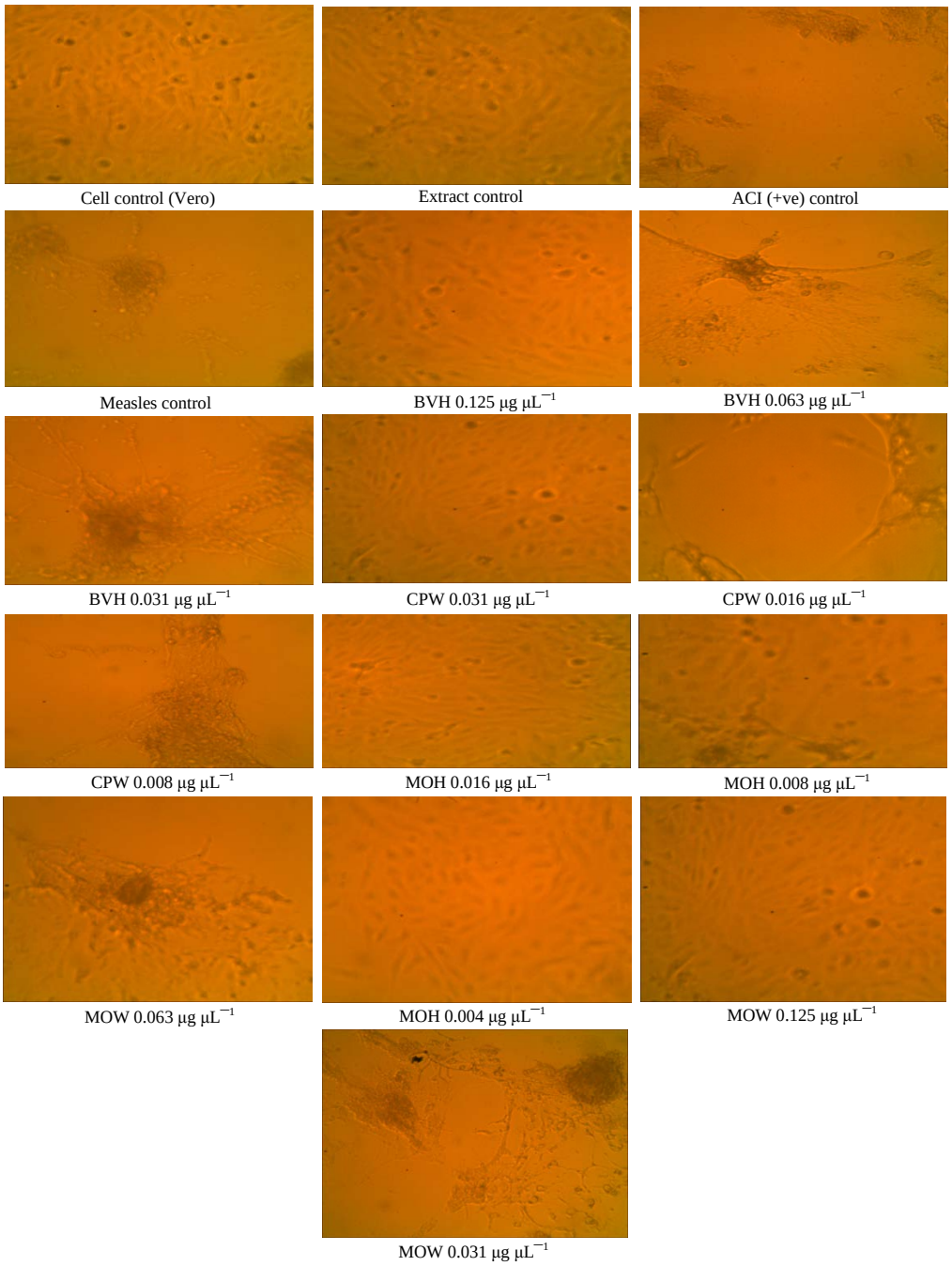

Fig. 1: Virucidal activity of the different extracts on 100 $TCID_{50}$ of measles virus (Magnification 100×)

glycoside (activity against RSV). Similarly five groups of biflavonoids (amentoflavone, agathisflavone, robustaflavone, rhusflavone and succedaneflavanone) are known to exhibit various antiviral effects against a number of viruses including respiratory viruses such as influenza A or B, parainfluenza type 3, measles etc. and herpes viruses (HSV-1, HSV 2, HCMV, VZV)[28]. Although tannins have been found to be toxic to some filamentous fungi, yeast and bacteria, they have also been observed to have an inhibitory action on viral reverse transcriptase[29]. Terpenes and terpenoids are reported to be active against bacteria, fungi, viruses and protozoa[27]. It has been reported that alkaloids may be useful against HIV infection as well as intestinal infections and other opportunistic infections associated with AIDS[30].

No antiviral medications are available for the treatment of measles although the virus has been reported to be susceptible to ribavirin[31]. This is confirmed in Fig. 1 and 2, where, aciclovir positive control drug failed to inhibit the virus

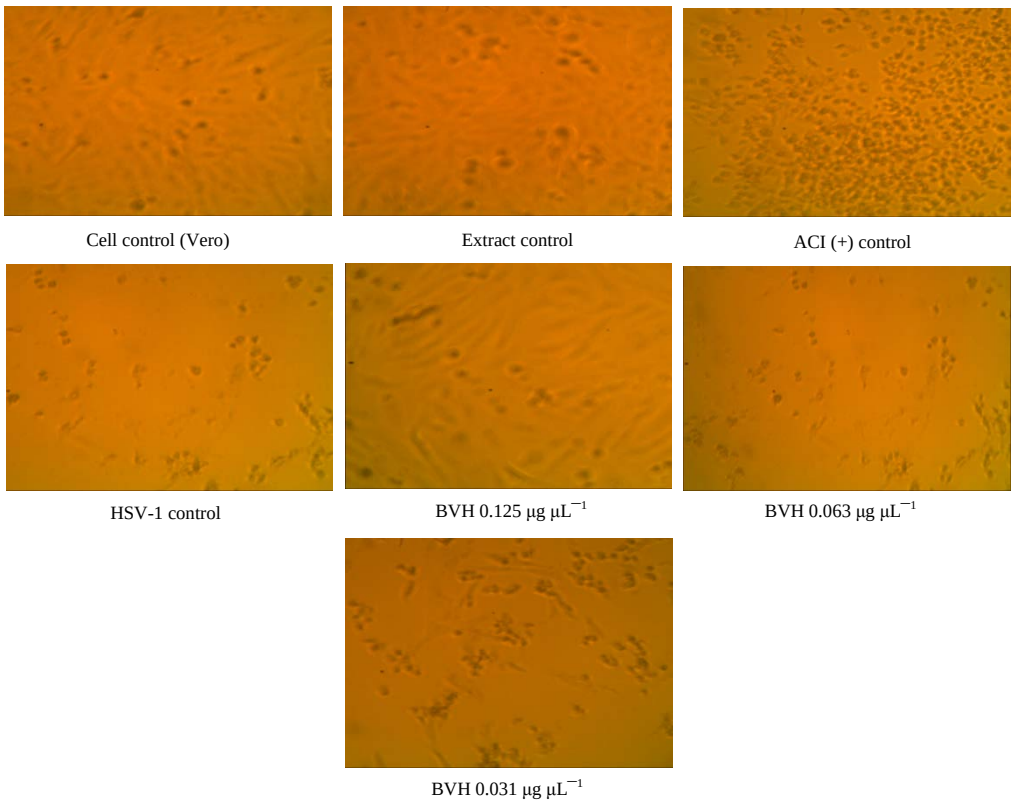

Fig. 2: Virucidal activity of BVH on 100 $TCID_{50}$ of HSV-1 (Magnification 100×)

where local herbs succeeded. Similarly there are no cures for HSV-1[32]. Antiviral medications commonly prescribed for treatment of HSV-1, which are prone to resistance by the virus. Only help lesions heal faster during an initial outbreak, lessen the frequency and duration of symptoms during recurrences, reduce the frequency of outbreaks and decrease viral shedding[33] and although these medications have proved effective, the increasing popularity of complementary and alternative treatments has led many HSV-1 positive individuals to experiment with the use of herbal and dietary supplements[33], thus confirming that *B. vulgaris* which inhibited HSV-1 in this study could also be a veritable alternative in the prevention and treatment of HSV-1 infection. This herb succeeded where Aciclovir, a known anti-HSV-1 drug, failed to inhibit the virus probably due to resistance of the virus to the drug. Similarly, in the absence modern orthodox treatment, evidences abound of plants different communities use in the treatment of measles[19,34,35,36]. These previous studies are thus confirming the findings of this study that herbs have been in the fore front of prevention and treatment of measles virus infection.

CONCLUSION

The extracts of the three plants, *Bambusa vulgaris*, *Citrus paridisi* and *Moringa oleifera* used in this study have shown significant antiviral activities against measles virus which is an enveloped RNA virus as well as HSV-1 which is also enveloped but belongs to the DNA class of viruses. This suggests that the extracts could be said to have a broad spectrum of activity against selected RNA and DNA viruses and so could serve as potential anti-measles virus and anti-HSV-1 agents. Further studies aimed at isolating and characterizing the active antiviral compounds from these three plants is therefore recommended. It is pertinent to suggest that, for the purpose of affordability in developing countries, to formulate these whole plants or their active constituents into drugs that could be used as antiviral agents.

REFERENCES

1. Bergner, P., 2005. Antiviral botanicals in herbal medicine. Med. Herbalism, 14: 1-12.
2. Davies, J., 2003. Anti-viral activity of plant extracts and herbs. Int. J. Trop. Med., 2: 49-54.

3. Afolayan, A.J. and T.O. Sunmonu, 2010. *In vivo* studies on antidiabetic plants used in South African herbal medicine. J. Clin. Biochem. Nutr., 47: 98-106.
4. FEPA., 1992. Nigeria: Country study report for Nigeria on costs-benefits and unmet needs of biological diversity conservation. National Biodiversity Unit, Lagos, Nigeria.
5. Karou, S.D., T. Tchacondo, D.P. Ilboudo and J. Simpore, 2011. Sub-saharan rubiaceae: A review of their traditional uses, phytochemistry and biological activities. Pak. J. Biol. Sci., 14: 149-169.
6. Barrett, T., 1999. Morbillivirus infections, with special emphasis on morbilliviruses of carnivores. Vet. Microbiol., 69: 3-13.
7. Griffin, D.E., 2001. Measles Virus. In: Fields Virology, Knipe, D.M., P.M. Howley (Eds.). Lippincott Williams and Wilkins, Philadelphia.
8. Jones-Engel, L., G.A. Engel, M.A. Schillaci, B. Lee, J. Heidrich, M. Chalise and R.C. Kyes, 2006. Considering human-primate transmission of measles virus through the prism of risk analysis. Am. J. Primantol., 68: 868-879.
9. Mossman, K.L. and J.R. Smiley, 2002. Herpes simplex virus ICP0 and ICP34.5 counteract distinct interferon-induced barriers to virus replication. J. Virol., 76: 1995-1998.
10. Baeten, J. and C. Celum, 2006. Herpes Simplex Virus and HIV-1. In: HIV in Site Knowledge Base, Eiperl, L., S. Coffey and P. Volberding (Eds.). Regents of the University of California, California, USA.
11. Khan, M.B., G. Ndaalio, M.H.H. Nkunya, H. Wevers and A.N. Sawhney, 1980. Studies on African medicinal plants. Part I. Preliminary screening of medicinal plants for antibacterial activity. Planta Medica, 40: 91-97.
12. Wang, L. and C.L. Weller, 2006. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Technol., 17: 300-312.
13. Somchit, M.N., R.A. Rashid, A. Abdullah, A. Zuraini and Z.A. Zakaria *et al*., 2010. *In vitro* antimicrobial activity of leaves of *Acalypha indica* Linn. (Euphorbiaceae). Afr. J. Microbiol. Res., 4: 2133-2136.
14. Patrick-Iwuanyanwu, K.C., E.N. Onyeike and A. Dar, 2011. Anti-inflammatory effect of crude methanolic extract and fractions of Ring worm plant *Senna alata* (L. Roxb) leaves from Nigeria. Der Pharmacia Sinica, 2: 9-16.
15. Beltran, O.M.S., 2008. Investigation of the Anti-mycobacterial and Cytotoxic Effect of Three Medicinal Plants Used in the Traditional Treatment of Tuberculosis in Northern Mexico and the Southwest United States. The University of Texas at El Paso, United States Pages: 61.
16. Evans, W.C., 2009. Trease and Evans Pharmacognosy. 15th Edn., Balliere Tindall, London, pp: 173-415.
17. Park, I.W., C. Han, X. Song, L.A. Green and T. Wang *et al.* 2009. Inhibition of HIV-1 entry by extracts derived from traditional Chinese medicinal herbal plants. BMC Complement. Altern. Med. 10.1186/1472-6882-9-29
18. Ojo, O.O., J.O. Oluyege and O. Famurewa, 2009. Antiviral properties of two Nigerian plants. Afr. J. Plant Sci., 3: 157-159.
19. Sunday, O.A., A.B. Munir, O.O. Akeeb, A.A. Bolanle and S.O. Badaru, 2010. Antiviral effect of *Hibiscus sabdariffa* and *Celosia argentea* on measles virus. Afr. J. Microbiol. Res., 4: 293-296.
20. Zandi, K., M. Taherzadeh, R. Yaghoubi, S. Tajbakhsh, Z. Rastian, M. Fouladvand and K. Sartavi, 2009. Antiviral activity of *Avicennia marina* against herpes simplex virus type 1 and vaccine strain of poliovirus (An *in vitro* study). J. Med. Plant Res., 3: 771-775.
21. Hierholzer, J.C. and R.A. Killington, 1996. Virus Isolation and Quantitation. In: Virology Methods Manual, Mahy, B.W.J. and H.O. Kangro (Eds.). Academic Press Limited, London, pp: 24-46.
22. Al-Jabri, A.A., M.D. Wigg, E. Elias, R. Lambkin, C.O. Mills and J.S. Oxford, 2000. *In vitro* anti-HIV-1 virucidal activity of tyrosine-conjugated tri-and dihydroxy bile salt derivatives. J. Antimicrob. Chemother., 45: 617-621.
23. McGeoch, D.J., F.J. Rixon and A.J. Davison, 2006. Topics in herpesvirus genomics and evolution. Virus Res., 117: 90-104.
24. Ghaemi, A., H. Soleimanjahi, P. Gill, E. Arefian, S. Soudi and Z. Hassan, 2009. *Echinacea purpurea* polysaccharide reduces the latency rate in herpes simplex virus type-1 infections. Intervirology, 52: 29-34.
25. De Clercq, E., 2005. Antiviral drug discovery and development: Where chemistry meets with biomedicine. Antiviral Res., 67: 56-75.
26. Stern, J.L., A.E. Hagerman, P.D. Steinberg and P.K. Mason, 1996. Phlorotannin-protein interactions. J. Chem. Ecol., 22: 1887-1899.
27. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.
28. Lin, Y.M., M.T. Flavin, R. Schure, F.C. Chen and R. Sidwell *et al*., 1999. Antiviral activities of biflavonoids. Planta Med., 65: 120-125.
29. Kaul, T.N., E. Middleton Jr. and P.L. Ogra, 1985. Antiviral effect of flavonoids on human viruses. J. Med. Virol., 15: 71-79.
30. McDevitt, J.T., D.M. Schneider, S.K. Katiyar and T.D. Edlind, 1996. Berberine: A candidate for the treatment of diarrhea in AIDS patients. Proceedings of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 5-18, 1996, Washington, DC., USA.
31. Plemper, R.K. and J.P. Snyder, 2009. Measles control-Can measles virus inhibitors make a difference? Curr. Opinion Invest. Drugs, 10: 811-820.

32. Leung, D.T. and S.L. Sacks, 2003. Current treatment options to prevent perinatal transmission of herpes simplex virus. Expert Opin. Pharmacother., 4: 1809-1819.
33. Haldiman, K.L., 2011. The use of herbal and dietary supplements in the treatment of herpes simplex virus. Herpes Herbal, June 14, 2011, pp: 1-14.
34. Obi, R.K., I.I. Iroagba and O.A. Ojiako, 2006. Virucidal potential of some edible Nigerian vegetables. Afr. J. Biotechnol., 5: 1785-1788.
35. Oladunmoye, M.K. and F.Y. Kehinde, 2011. Ethnobotanical survey of medicinal plants used in treating viral infections among Yoruba tribe of South Western Nigeria. Afr. J. Microbiol. Res., 5: 2991-3004.
36. Nwachukwu, C.U., B.C. Ijioma and E.O. Onwukwe, 2011. Medicinal plants and their correlate use by the inhabitants of Ikeduru LGA of Imo State Nigeria. Int. J. Res. Rev. Applied Sci., 8: 123-126.