Susceptibility of Two RNA Viruses of Public Health Significance to Selected Nigerian Medicinal Plants

ObiR.K'and Shenge J.A

1. Department of Microbiology, Federal University of Technology, Owerri, Irno State, Nigeria

2. Department of Virology, College of Medicine, University of Ubadan, Oyo State, Nigeria

Abstract: *Background/Aim:* The lack of effective therapies and/or vaccines for several viral infections, and the rapid emergence of new drug-resistant viruses have necessitated the need for developing new and effective antiviral agents. In this study we evaluate the antiviral potentials of three plants indigenous to Nigeria on vaccine strains of Yellow fever virus (YFV) and Polio virus (PV) in order to inform their usefulness in antiviral drug design. Fresh leaves of *Bambusa vulgaris* Schrad., *Moringa oleifera* (L), and seed of *Citrus paridisi* Macfad were collected from Lagos State, South Western Nigeria. Extraction of the plant materials was done with analar grade methanol using the Soxhlet extractor and concentrated using the rotary evaporator. Results showed that the crude extracts of the three plants inhibited YFV *in vitro*, with the virus being more susceptible to *C. paridisi* Macfad at all the concentrations tested. *B. vulgaris* Schrad., and *M. oleifera* (L) inhibited the virus at two concentrations of 0.031 and 0.016 ug \xh'\ andO.063 and 0.031 ug uL~',respectively. PV was resistant to all the extracts at all the concentrations. Results of pre- and post- infection antiviral activities of the extracts on the replicative cycle of the viruses showed both adsorption/entry, and post infection inhibitors. Phytochemical screening of the extracts showed the presence of terpenes, alkaloids, flavonoid, tannins, combined and free anthraquinones, cardiac glycosides, and saponins. This study revealed that some Nigerian medicinal plants could serve as alternative agents for treating and/or preventing infections caused by RNA viruses.

Key words: Yellow fever virus, polio virus, *Bambusa vulgaris, Moringa oleifera, Citrus paridisi,* antiviral drugs

Introduction

Viral infections play an important role in human diseases (Kitazato *et al.*, 2007).Recent outbreaks in the advent of globalization and ease of travel have underlined their prevention as a critical issue in safeguarding public health. Despite the progress made in immunization and drug development, many viruses lack preventive vaccines and efficient antiviral therapies, which are often beset by the generation of viral escape mutants. Thus, identifying novel antiviral drugs is of critical importance and natural products are an excellent potential source for such discoveries (Liang-Tzung *et al.*, 2014).

Several hundred natural active compounds have been identified worldwide (Newman *et al.*, 2007). Many of them have complementary and overlapping mechanisms of action, either inhibiting synthesis of the viral genome, viral protein or blocking of viral replication. These natural active compounds, which contain more characteristics of high chemical diversity and biochemical specificity than standard combinatorial formula, offer major opportunities for finding novel structures that are active against a wide range of assay targets. In addition, natural products that are biologically active in assays are generally small molecules with drug-like properties. They are capable of being absorbed and metabolized by the body. Hence, the cost of producing orally active medicines are likely to be much lower than that of biotechnological products or most compounds produced to date from combinatorial chemistry. Therefore, natural products, including traditional medicinal plants (herbs), offer great promise as potentially effective new antiviral drugs (Kitazato *et al.*, 2007).

The continued discovery and development of new formulations of herbal medicines, containing a combination of multiple ingredients that synergistically act to potently and selectively inhibit replication of viruses of public health significance like yellow fever and polio virus at different stages and strengthen the impaired immune system, should be a potential therapeutic option in the future (Kitazato *et al.*, 2007).

Yellow fever is caused by the yellow fever virus, a 40- to 50-nm-wide enveloped positive-sense spherical single stranded RNA virus, belonging to the family *Flaviviridae* and genus *Flavivirus* (Stock *et al.*, 2013). This arthropod-borne pathogen is the causative agent of severe and fatal disease in humans and has a considerable socioeconomic effect worldwide (Stock *et al.*, 2013).

Poliovirus is a human enterovirus and member of the family of Picornaviridae (Ryan and Ray, 2004; Carstens and Ball, 2009). It is a naked single stranded (+) sense RNA icosahedral virus (WHO, 2015). Owing to the expression of three unique sets of four different neutralization antigenic determinants on the poliovirion surface referred to as N-Ag1, 2, 3A, and 3B (Lee *et al.*, 1977), poliovirus occurs in three serotypes, termed types 1, 2, and 3, where the names Mahoney or Brunhilde, MEF-1/Lansing, and Saukett/Leon designate a strain of each serotype, respectively (Minor, 1990). Poliomyelitis caused by polio virus is a paralyzing

*Corresponding author:

*obi.robert@ymail.com * Obi R.K*

disease transmitted primarily through person-to-person spread through the fecal-oral route (WHO, 2015).

In this study, we investigated the antiviral potentials of three locally available herbs in Nigeria against known viruses of public health significance in Nigeria, namely Yellow Fever virus and Polio virus

Materials and Methods

Study Design: This is an *in vitro* tissue culture study Vero cell lines in MEM medium in 96 well tissue culture plates. The study involved virucidal, and pre- and post- infection antiviral activities of the extracts on the test viruses.

Study site: 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* Schrad. and *M. oleifera* (L) and seeds of *C. paridisi* Macfad. were collected from Okota, Lagos, South Western Nigeria. The plants materials were carefully examined and 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. The voucher numbers were *B. vulgaris* Schrad. (LUH 5493), *C. paradisi* Macfad. (LUH 7659), and *M. oleifera* (L)(LUH 5498).

Extraction of plant materials

The method of extraction of plant materials was as described by Wang and Weller, (18). Briefly the leaves were dried, blended to a fine powder, and subjected to Soxhlet extraction (Nahalito) using 99% Analar grade methanol. They were concentrated *in vacuo* at 45°C to a final volume of 3 mL using a rotary evaporator (Buchi). The solid residues obtained after evapporation were dried, weighed and preserved at -20°C in airtight sterile McCartney bottles till further use as recommended by Somchit *et al*., (2010) and Patrick-Iwuanyanwu *et al*., (2011).

Reconstitution of extract: About 10 (1 mg/ml) mg of each pasty solid plant extract was weighed and introduced into sterile calibrated centrifuge tubes. They were then reconstituted in 0.5% dimethyl sulfoxide (DMSO) (Sigma) and shaken vigorously to ensure complete dissolution. They were then brought to a final volume of 10 mL with the addition of 9.95 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 as recommended by Beltran, (21).

Phytochemical screening

The extracts were first reconstituted in methanol extraction solvent and then tested by standard phytochemical methods according to Evans, (2009) 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 as recommended by In-Woo *et al*., (2009), Ojo *et al*., 2009; and Omilabu *et al*., 2010. Briefly Vero cells were prepared at a density of 8 $x10^4$ cells mL^{-1} in a 10% MEM in 75 cm^2 tissue culture flasks (Cell Treat, USA). A 100 µL of this cell suspension (containing 8000 cells) was then dispensed into each well of a flat bottomed 96-well tissue culture plate (Cell Treat, USA) and incubated for 24h at 37°C. The 10% MEM was aspirated thereafter and discarded. Then eight sterile universal bottles labeled with the code name for each extract was arranged on a rack and a 2-fold serial dilution was carried out. The positive control, Virest 200 mg, a brand of Acyclovir (Hovid Bhd, Malaysia), was prepared by dissolving 200 mg of the tablet in 200 mL of Phosphate buffered saline (PBS) and filtered using 0.22µm of 250 mL (Nalgene filter, USA). Cell viability was monitored every day for 14 days for any possible immediate changes in morphology (CPE) compared with the control wells containing only medium and no extract, using an inverted microscope (Inverskop 40C).

Isolation of test viruses

Polio (types 1,2&3, Serum Institute, Hadaraba, Pune, India), and Yellow Fever (17D strain, FSUE of Chimakov IPVE, Russian Acad. Med. Sci.) viruses were isolated from the respective vaccines of the viruses obtained from Institute of Child Health, University College Hospital (UCH), Ibadan, The viruses were titrated and using Reed-Muench method, the tissue culture infective doses of the viruses ($TCID_{50}$ ml^{-1}) were calculated to be $10^{-6.3}$ for Yellow Fever virus and $10^{-6.5}$ for Polio viruses. The respective $100TCID_{50}$ for both viruses which was used for the screening was $10^{-4.3}$ and $10^{-4.5}$.

Test for virucidal activity: Vero cells were prepared at a density of 8 $x10^4$ cells ml^{-1} in a 10% MEM in $75cm^3$ tissue culture flasks (Cell Treat, USA).Hundred microlitre of this cell suspension (containing 8000 cells)

was then dispensed into each well of a flat bottomed 96-well tissue culture plate (Cell Treat, USA) and incubated for 24h at 37°C in 5% CO_2 and moisture. Then (a) Hundred microlitre of 100 $TCID_{50}$ virus titer + 100µL of minimum non-toxic concentration of the test extract in graded concentrations, and (b) 100µL of 100 $TCID_{50}$ virus titer + 100 µL of 1% MEM as control were prepared in a plain bottle and incubated for 1 h at 37°C in 5% CO_2. Also prepared were 100µLof acyclovir positive control + 100µL of 100 $TCID_{50}$ virus titer. The 10% MEM in the 96 - well tissue culture plate was aspirated and discarded ((Zandi *et al.*, 2009)).

After the 1h incubation period, 100µL of (a) virus + extract mixture was inoculated in triplicate unto the 96-well tissue culture plate seeded with Vero cells. Similarly 100µL of (b) virus + 1% MEM mixture was added in triplicate to thelast three wells of each row on the same 96-well tissue culture plate to serve as control. Also added in triplicate were 100µLof acyclovir positive control + 100µL of virus. The last two rows of wells were kept for cell control and extract/fraction control. All the mixtures were incubated at 37°C in 5% CO_2 and moisture (Zandi *et al.*, 2009; Omilabu *et al.*, 2010).

The setup was monitored every day under the inverted microscope (Inverskop 40C) for 7 days and scored **for** the presence of virus-induced syncytia as follows: 4⁺ for complete (100%) CPE, 3⁺ for 75%, 2⁺ for 50%, 1⁺ for 25%, and 0, when there was no CPE (Hierholzer and Killington, 1996; Omilabu *et al.*, 2010). The wells containing virus and extracts were also scored and compared with the wells containing virus but no compound and wells containing virus and acyclovir. The wells containing virus and extract were also scored and compared with the wells containing virus but no extract (Aljabri *et al.*, 2000)).

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 (Hierholzer and Killington, 1996 and Omilabu *et al.*, (2010).

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 (Hierholzer and Killington, 1996 and Omilabu *et al.*, 2010).

Results

Results of the phytochemical screening showed the presence of classes of bioactive phytochemicals in the plant extracts. Terpene was very abundant in *M. oleifera* (L), while absent in *C. paradisi* Macfad. and *B. vulgaris* Schrad. as shown in Table 1.

As part of ethnopharmacological studies of medicinal plants, the inclusion of cytotoxicity and other toxicity protocols in the study are useful in detecting potential toxicity (Hanisa *et al.*, 2014) and the standard is to select extract concentrations below the toxic level for antiviral screening. At the end of the period of incubation, the results of the toxicity evaluation of the extracts were as shown in Figure. The most toxic extract was from *B.* vulgaris Schrad., with IC_{50} of 0.065 µg $µL^{-1}$, while the least was Acyclovir with IC_{50}of4.56 µg $µL^{-1}$. The $IC_{50}$of the extracts was observed to be higher than their maximum non-toxic concentrations (MNTC). However to show that the extraction solvent (methanol) made no contribution to the cytotoxicity of the extracts, there was 100% CPE recorded on all the wells of the solvent control, while non was observed on the wells containing cells and extracts only.

The aim of virucidal activity was to determine whether the extract could inactivate the virus before both were incubated with the cell (Fayad *et al.*, 2013). Result of the virucidal activity of the extracts on the test viruses was as shown in Table 2. YFV was susceptible to all the extracts with *C. paridisi* Macfad. being more potent than *B. vulgaris* Schrad. and *M. oleifera* (L), having inhibited the virus at all the concentrations tested. On the other hand, PV was resistant to all the extracts at all the concentrations (Table 3).The result in Figure 2 showed the percentage inhibition of YFV by the methanol extracts. The result showed that *M. oleifera* (L) with 20.8% inhibition of the virus was the least sensitive, while *C. paridisi* Macfad., with 5.21% was the most susceptible. Plate 1 showed the photomicrograph of the extracts that inhibited YFV. The rounding of the cells were indicative of the characteristic CPE of YFV.

Pre- and post- infection was done to determine the mechanism of action of the plant extracts as it concerns replication cycle of the viruses (Jaime *et al.*, 2013). Table 4 shows the result of the mechanism of action of the extracts on the test viruses. All the extracts that inhibited YFV were active at the post- infection assay, while the virus was resistant to them at the pre-infection level. PV was still resistant to the extracts at both the pre- and post- infection inhibitory assays.

The potential value of a compound can be assessed by determining the ratio of its cell toxicity to its antiviral activity. This ratio is known as the selectivity index (SI) (Driscoll, 2002). The result in Table 5 showed the inhibitory concentration (IC_{50}) and selectivity index (SI) of the extracts that inhibited YFV. All the extracts showed SI which were all above their IC_{50}. The SI were also observed to be above one, showing that they are less toxic and could be used as a pharmaceutical raw materials for drug production.

Table 1 Phytochemical screening of the methanol extract of plant materials

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

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

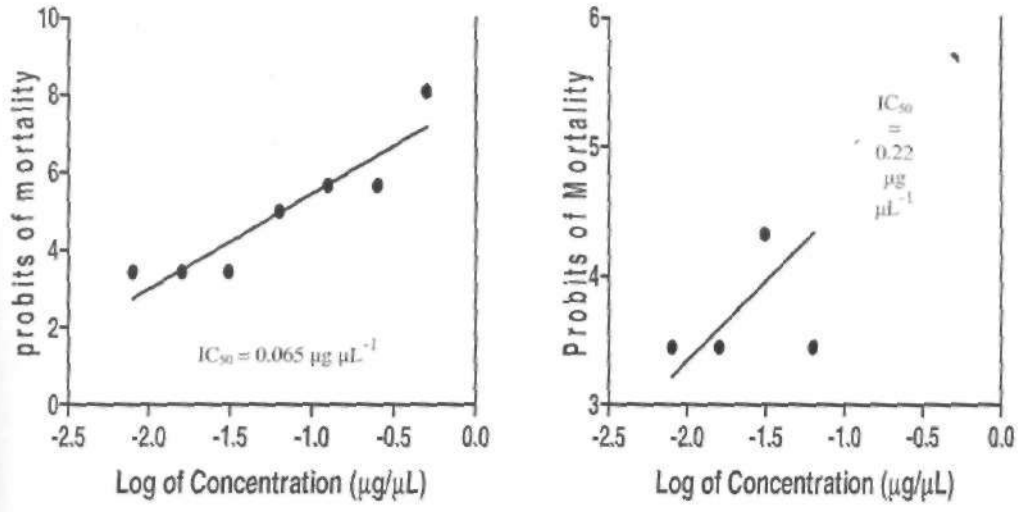

Fig. 1. Probit analyses and IC_{50} of the toxicity profile of methanol extracts of (a) *B. vulgaris* (b) *C. paradisi* (c) *M. oleifera* (d) Acyclovir

Table 2 Virucidal activity of the plant extracts (µg µL^{-1}) against 100 $TCID_{50}$ of YFV

Extract	Extract concentration (µg µL^{-1})									
	1	0.5	0.25	0.125	0.063	0.031	0.016	0.008	0.004	0.002
	Bambusa vulgaris									
BVM						0	0	1^+		
	Citrus paradisi									
CPM						0	0	0		
	Moringa oleifera									
MOM					0	0	3^+			
	Acyclovir									
ACI					4^+	4^+	4^+			
Cell con					0	0	0			
Ext con.					0	0	0			

4+=Complete (100%) cytopathic effect (CPE); 3^+=75% CPE; 2^+=50% CPE; 1^+=25% CPE; 0=No CPE
Key: BVM *B. vulgaris* methanol CPM *C. paridisi* methanol MOM *M. oleifera* methanol

Table 3 Virucidal activity of the plant extracts (µg/µl) against 100 $TCID_{50}$ of Polio viruses

Extract	Extract concentration (µg µL^{-1})									
	1	0.5	0.25	0.125	0.063	0.031	0.016	0.008	0.004	0.002
	Bambusa vulgaris									
BVM						4^+	4^+	4^+		
	Citrus paradisi									
CPM						4^+	4^+	4^+		
	Moringa oleifera									
MOM					4^+	4^+	4^+			
	Acyclovir									
ACI					4^+	4^+	4^+			
Cell con					*0*	*0*	*0*			
Ext con					0	0	0			

4+=Complete (100%) cytopathic effect (CPE); 3^+=75% CPE; 2^+=50% CPE; 1^+=25% CPE; 0=No CPE
Key: BVM *B. vulgaris* methanol CPM *C. paridisi* methanol MOM *M. oleifera* methanol

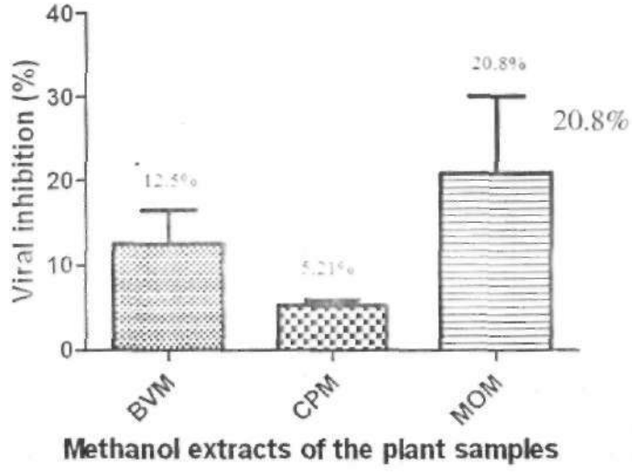

Fig 2. Percentage inhibition of YFV by methanol extracts of *B. vulgaris*, *C. paridisi*, and *M. oleifera*

Plate 1 Virucidal activity of the different extracts on 100TCID$_{50}$ of Yellow Fever virus *(Magnification X100)*

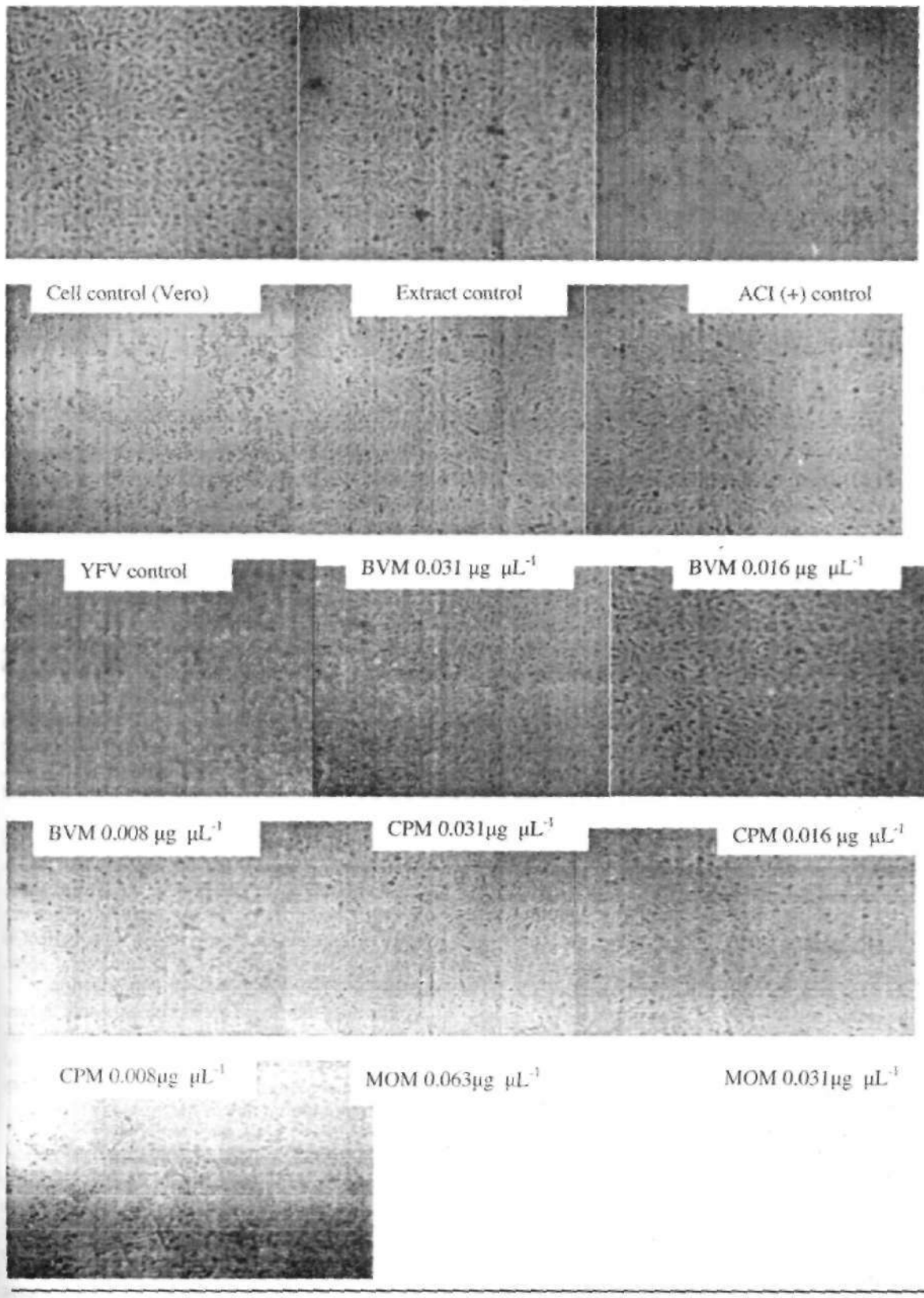

Table 4.Pre- and post- infection inhibitory tests of the extracts on 100 $TCID_{50}$ of YFV and PV

	Pre infection Antiviral Activity						Post Infection Antiviral Activity					
	Extract concentration (µg µL^{-1})											
Extract	0.125	0.063	0.031	0.016	0.008	0.004	0.125	0.063	0.031	0.016	0.008	0.004
	YFV											
BVM			4^{+}	4^{+}	4^{+}				0	0	1+	
CPM			4^{+}	4^{+}	4^{+}				0	0	0	
MOM		4^{+}	4^{+}	4^{+}				0	0	3+		
	PV											
BVM			4^{+}	4^{+}	4^{+}				4^{+}	4^{+}	4^{+}	
CPM			4^{+}	4^{+}	4^{+}				4^{+}	4^{+}	4^{+}	
MOM		4^{+}	4^{+}	4^{+}				4^{+}	4^{+}	4^{+}		

Table 5. 50% inhibitory concentration (IC_{50}) and selectivity index (SI) of extracts that inhibited measles virus

Extract	Vero cell IC_{50} (µg µL^{-1})	Virus Replication IC_{50} (µg µL^{-1})	SI
	VIRUCIDAL ACTIVITY		
	YFV		
BVM	0.065	0.004	16.3
CPM	0.22	0.002	110.0
MOM	0.313	0.002	156.5

Discussion

The lack of effective therapies and/or vaccines for several viral infections, and the rapid emergence of new drug-resistant viruses have indeed necessitated a growing need for developing new and effective chemotherapeutic agents to treat viral diseases (Kitazato *et al.*, 2007).

The current study started with the evaluation of the toxicity of the crude extracts on Vero cells. The $IC_{50}$of the extracts revealed them as potential candidates for drug development against susceptible viruses. This was because the extracts displayed IC_{50} values below that recommended by the National Cancer Institute (NCI) for crude extracts (Fig. 1). The recommended guideline set the IC_{50}limit of activity for crude plant extracts at < 20 µg/mL after the exposure time of 72 hours (Abdel-Hammed *et al.*, 2012). According to Mahavorasirikul *et al* (2010), the lower the IC_{50} values of a given crude extract, the higher will be its toxicity potential. This assertion was in agreement with the findings of this present study which shows that the $IC_{50}$of 4.56 µg/µL observed for Acyclovir, a processed and purified drug, was far higher than that recorded for all the crude extracts.

The antiviral activities shown in Tables 2, 3 and 4, revealed that *B. vulgaris* Schrad., *C. paridisi* Macfad. and *M. oleifera* (L) could be alternative treatment options for infections due to 100$TCID_{50}$of YFV. This was because the extracts were able to inactivate the virus in the virucidal and post- infection assays. PV on the other hand, was resistant to the extracts at all the concentrations tested, probably because the three strains of the virus present in the vaccine used for virus isolation, may have acted in concert to inhibit the antiviral activities of the extracts. In similar studies Ojo *et al.*, (2009) confirmed the findings of this study that *B. vulgaris* Schrad. was inactive against PV, while active against YFV. Other workers had earlier reported positive results with other herbs against YFV (Esimone *et al.*, 2007; Fasola *et al.*, 2011), thus corroborating the results of the present study that Nigerian herbs could become veritable alternatives in the global effort to manage this dangerous viral haemorrhagic infection.

The abundant phytochemicals present in the tissues of the crude extracts of the three plants were as shown in Table 1.These identified phytochemicals have been variously reported as being antimicrobial (Cowan, 2009), and could have been responsible for the antiviral activities of the extracts against YFV. The selectivity index (SI) of all the extracts used in this study as revealed in Table 5 shows that the extracts are safe to be used as antiviral agents since their values are above the IC_{50} (De Clercq, 2005) of the extracts. When the IC_{50}values of extracts is below its SI, that extract is considered safe for use, directly as antimicrobial agent, or indirectly as a raw material for drug production.

The fact that there are no medications against viral infections, or that those currently available are prone to resistance by the viruses, was again manifested in this study where Acyclovir, a known drug for herpes viruses, could not inhibit any of the viruses.

Conclusion

Bambusa Vulgaris Schrad., *Citrus paradise* Macfad., and *Moringa oleifera* (L) have shown promise, in this study, to be potential anti-YFV agents. While work continues to find functional alternatives for

PV, it is absolutely necessary that further studies be conducted to isolate the active components responsible for the anti-YFV activities of these plants. This could be a cost effective way of managing this fatal viral haemorrhagic infection, and reduce over dependence on vaccines have sometimes failed to protect, due to shortage, poor coverage, and vaccine failure.

References

Abdel-Hameed E S, Salih A. Bazaid S A, Shohayeb M M, El-Sayed M M, El-Wakil E A (2012). Phyto chemical studies and evaluation of antioxidant, anticancer and antimicrobial Properties of Conocarpus erectus L. growing in Taif, Saudi Arabia. *Eur J Med Plants*; **2**: 93-112.

Al-Jabri A A, Wigg M D, Elias E, Lambkin R, Mills CO and Oxford JS (2000). *In vitro* anti- HIV-1 virucidal activity of tyrosine-conjugated tri- and dihydroxy bile salt derivatives. *Journal of Antimicrobial* **45**(5): 617-621

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, AAT 1456752. http://proquest.umi.com/pqdlink?did=1594487581&Fmt=7&clientId=79356&RQT=309&VName=PQD

Carstens E B and Ball L A (2009). Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses. *Archives of Virology* 154 (7): 1181-1188 Cowan MM (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*, 12: 564-582

De Clercq E (2005). Antiviral drug discovery and development: where chemistry meets with biomedicine *Antiviral Research*, **67**: 56–75

Driscoll J S (2002). *Antiviral Drugs*. Ashgate Flint S J (2004) Chapter 19 in *Principles of Virology: Molecular Biology, Pathogenesis and Control of Animal Viruses*, 2nd edn, ASM Press

Esimone C O, Ofokansi K C, Adikwu M U, Ibezim E C, Abonyi D O, Odaibo G N, Olaleye D O (2007). *In vitro* evaluation of the antiviral activity of extracts from the lichen *Parmelia perlata* (L.) Ach. against three RNA viruses. *J Infect Developing Countries*; **1**(3): 315-320.

Evans W C (2009). *Trease and Evans Pharmacognosy* 15th Edition. Sunders Elsevier, UK London. Pp 173-415.

Fasola T R, Adeyemo F A, Adeniji J A, Okonko I O (2011). Antiviral Potentials of *Gossypium hirsutum* Extracts on Yellow Fever Virus. *New York Science Journal*; **4**(10):30-35.

Fayyad A, Ibrahim N, Yaacob W A (2013). *In vitro* Virucidal Activity of Hexane Fraction of *Marrubium vulgare* Against Type 1 Herpes simplex Virus. *American Journal of Drug Discovery and Development*, **3**: 84-94.

Hanisa H, Mohdazmi M L, Suhaila M, Hakim M N (2014). effects of *Centella asiatica* L., *Curcuma longa* L., and *Strobilant hescrispus* L. extracts on 3 kidney cell lines: *in vitro* cytotoxicity analysis. *International Journal of Pharmacy and Pharmaceatical Sciences*; **6**(2): 388-392

Hierholzer J C and Killington R A (1996). *Virus isolation and quantitation* In *Virology Methods Manual*, Mahy BWJ and Kangro HO (ed). Academic Press Limited, 24-28 Oval Road, London NW1 7DX; pp 24-46

In-Woo P, Changri H, Xiaoping S, Linden A, Ting Y, Ying L, Changchun C, Xinming S, Biao Y, Guanying C, Johnny JH (2009). Inhibition of HIV-1 entery by extracts Derived from Chinese medicinal herbal plants. *BMC Complementary and Alternative Medicine***9**:29-34

Jaime M F V, Redko F, Muschietti L V, Campos R H, Mrtino V S, Cavallaro R V (2013). *In Vitro* antiviral activity of plant extracts from Asteraceae medicinal plants. *Virol. J.* **10**

Kitazato K, Wang Y, Kobayashi N (2007). Viral infectious diseases and natural products with Antiviral activity. *Drug Discover. Ther.*; **1**: 14-22

Lee Y F, Nomoto A, Detjen B M, Wimmer E (1977). A protein covalently linked to poliovirus genome RNA. *Proc Natl Acad Sei USA*, **74**:59-63

Liang-Tzung Lin, Wen-Chan Hsu, and Chun-Ching Lin(2014). Antiviral Natural Products and Herbal Medicines. *J Tradit Complement Med.*: 4(1):24–35.

Mahavorasirikul W, Viyanant V, Chaijaroenkul W, Itharat A, Na-Bangchang K (2010). Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells in vitro. *BMC Complement Altern Med*; 10:55.

Minor P D (1990). Antigenic structure of picornaviruses. *Curr Top Microbiol Immunol*, **161**:121-154.

Newman D. J, Cragg G. M, Snader K. M (2003). Natural products as sources of new drugs over the period 1981–2002. *J Nat Prod.* 66: 1022–1037.

Ojo O. O, Oluyege J. O, Famurewa O (2009). Antiviral properties of two Nigerian plants. African *Journal of Plant Science;*3(7): 157-159

Omilabu S A, Munir A B, Akeeb O O, Adesanya A B, Badaru SO (2010). Antiviral effect of *Hibiscus sabdariffa* and *Celosia argentea* on measles virus. *African Journal of Microbiology Research*4 (4): 293-296

Patrick-Iwuanyanwu K C, Onyeike Eugene N and Dar A (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** (4): 9-16

Ryan KJ and Ray CG (eds.) (2004). *Enteroviruses. Sherris Medical Microbiology* (4th ed.). McGraw Hill. pp. 535–7.

Somchit M N, Reezal I, Nur I E, Mutalib A R (2003). In vitro antimicrobial activity of ethanol and water extracts of *Cassia alata. J Ethnopharmacol*; 84(1):1-4.

Stock N K, Laraway H, Faye O, Diallo M, Niedrig M, Sall A A (2013). Biological and Phylogenetic Characteristics of Yellow Fever Virus Lineages from West Africa. *Virol.***87**(5):2895-2907.

Wang L and Weller C L (2006). Recent advances in extraction of nutriceuticals from plants *Trends in Food Science & Technology***17**: 300–312.

WHO (2015). Poliomyelitis. Fact sheet 114 Number http://www.who.int/mediacentre/factsheets/fs114/en/ (Accessed 17th December, 2015).

Zandi K, Taherzadeh M, Yaghoubi R, Tajbakhsh S, Rastian Z, Fouladvand M and Kohzad S (2009). Antiviral activity of *Avicennia marina* against herpes simplex virus type 1 and vaccine strain of poliovirus (An *in vitro* study). *Journal of Medicinal Plants Research***3**(10):771-775