Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Microbiology

ISSN 1816-4935
DOI: 10.3923/jm.2018.138.146

Research Article
In vitro Antiviral Activities of *Bryophyllum pinnatum* (*Odaa opuo*) and *Viscum album* (Awuruse)

[1]Robert Kelechi Obi and [2]Juliet Adamma Shenge

[1]Department of Microbiology, School of Biological Sciences, Federal University of Technology, Owerri, Imo State, Nigeria
[2]Department of Virology, College of Medicine, University of Ibadan, Nigeria

Abstract

Background and Objective: Despite tremendous progress in human medicine, no drugs exist for the complete treatment of viral diseases. This study was designed to investigate the antiviral potentials of two medicinal plants available locally in Lagos, South Western, Nigeria. **Materials and Methods:** Fresh leaves of *Bryophyllum pinnatum* (L.) and *Viscum album* (L.) were collected from Owerri and its environs. They were air-dried for seven days and extraction was done with methanol using the Soxhlet extractor. Concentration of the samples was done using the rotary evaporator. Measles (MV), polio PV and yellow fever (YFV) viruses were isolated from their respective vaccines, while herpes simplex virus-1 (HSV-1) was isolated from a positive HSV-1 male case. **Results:** The toxicity profile showed that the maximum non-toxic concentration (MNTC) of *B. pinnatum* (L.) was 0.016 μg μL^{-1} with an IC_{50} of 0.063 μg μL^{-1} while that of *V. album* (L.) was 0.063 μg μL^{-1} and IC_{50} of 0.313 μg μL^{-1}. Result of the antiviral analysis showed that two of the viruses were susceptible to *B. pinnatum* (L.) and *V. album* (L.). While HSV-1 was sensitive to *B. pinnatum* (L.) at the concentrations of 0.016 μg μL^{-1} (IC_{50} 0.004 μg μL^{-1}), MV was susceptible to *V. album* (L.) at 0.063 μg μL^{-1} (IC_{50} 0.031 μg μL^{-1}) and 0.031 μg μL^{-1} (IC_{50} 0.039 μg μL^{-1}). The PV and YFV were resistant to both plants extracts at the concentration same concentrations of 0.016 μg μL^{-1}. Result of the phytochemical analysis of both plants showed the presence of various secondary useful metabolites. **Conclusion:** This study has shown that the solution to the world-wide menace of MV and HSV-1 viral diseases could be found in the forest zones of Nigeria.

Key words: Measles virus, polio virus, yellow fever virus, herpes simplex virus, medicinal plants, lagos

Citation: Robert Kelechi Obi and Juliet Adamma Shenge, 2018. *In vitro* antiviral activities of *Bryophyllum pinnatum* (*Odaa opuo*) and *Viscum album* (awuruse). Res. J. Microbiol., 13: 138-146.

Corresponding Author: Robert Kelechi Obi, Department of Microbiology, School of Biological Sciences, Federal University of Technology, Owerri, Imo State, Nigeria Tel: 234-08038757515

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

Viral diseases are serious health challenges which absolutely cannot be ignored. The emergence of drug resistance and spread of new viruses are challenges posed by most viral diseases. To develop new antiviral agents with safe properties, significant effect and selective toxicity remains a pressing need of humans globally. Due to numerous advantages which include constant availability of plant resources, low price and less adverse effect, medicinal plants have become the research focus in antiviral designs. In recent years, there are numerous studies ranging from separation of active ingredients to antiviral mechanism or mode of action[1].

The advantages of natural compounds include fewer side effects in comparison to orthodox medicine and the production of synergistic effects for a more positive treatment outcome. However, the potential benefits of these natural compounds need to be confirmed in large, rigorous trials. The continued discovery and development of new formulations from medicinal plants, containing a combination of multiple ingredients that synergistically act to selectively inhibit virus replication at different stages and strengthen the impaired immune system, should be a potential therapeutic option in the future[2]. This has prompted research into the antiviral activities of crude extracts of several plants available in our locality.

Bryophyllum pinnatum (L.) is widely distributed throughout the continent of Africa, especially in warm and dry areas as well as on rocky surfaces with little water[3]. The plant could also be found in temperate regions of Asia, the Pacific and Caribbean[4]. The *B. pinnatum* (L.), a flowering plant in the *Crassulaceae* family, is a glabrous, laxly erect, fleshy shrub 60-120 cm tall and branches from the base. The leaves, about 10 cm long and 5-6 cm wide are opposite, in pairs, simple at the lower end and pinnate, rounded and large at the upper end. The margins of the leaves bear curved crenations (notches), with regular, blunt or rounded margins, which sometimes bear small plantlets or bulbils. The flowers which form nodules in terminal panicles are greenish-yellow but purplish at the base[3]. The fruit is a follicle with many seeds[5].

Viscum album (L.) or Mistletoe, comprising of several species of flowering plants in the order Santalales and family Santalaceae (Viscaceae), is an evergreen hemi-parasitic shrub, which grows up to 1 m high, with numerous, regularly forked branches and stems 30-100 cm long. The leaves, yellowish-green in colour are in pairs and opposite, widest above the middle, narrowly oval, slightly curved, entire and leathery and 2-8 cm long and 0.8-2.5 cm broad. The flowers, yellowish-green in colour are very small and inconspicuous, 2-3 mm in diameter. The fruit is a white or yellow berry containing one (very rarely several) seed embedded in a very sticky, glutinous fruit pulp[6]. Mistletoe grows as a hanging bush on the branches of a wide range of host trees such as pines, citrus, avocado, oaks, apple trees, African oil bean and commonly reduces their growth or kills them with heavy infestation[7]. It is possible that pharmacologically active compounds may pass from the different host trees to the parasitic plants[8].

Viruses cause a variety of illnesses in humans, yet only a few antiviral drugs have been developed. It has therefore become imperative, in sourcing for solutions from local sources, to investigate two plants *B. pinatum* (L.) and *V. album* (L.), known for ages for their medicinal qualities in traditional medicine, for a possible antiviral activity against a broad class of viruses, including measles, polio, yellow fever and herpes simplex virus.

Viral infections are indeed a continuing danger to everyone regardless of age, sex, lifestyle, ethnic background and socioeconomic status. Fortunately, while there are few effective antiviral drugs available, there are many plant extracts with demonstrable antiviral activities[9]. This informed the current study, aimed at exploring and evaluating the antiviral properties of extracts of some edible plants, known for their long use in traditional medicine in Nigeria as they could become veritable sources of modern pharmaceutical drug development for the treatment of such viral infections like Measles, Polio, Yellow fever and Herpes.

MATERIALS AND METHODS

Study design/study site: This was an *in vitro* tissue culture study using Vero cell lines in minimum essential medium (MEM) in 96 well tissue culture plates. The toxicity evaluation of the extracts was carried out and their maximum non-toxic concentration (MNTC) selected for antiviral studies. There was virucidal activity, adsorption and post-adsorption assays of the extracts on the test viruses.

Study site: Initial tissue culture study was carried out at the Virology Laboratory in the Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos. Extraction of plant materials was done at the Pharmacognosy Department, Faculty of Pharmacy, University of Lagos. 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. pinatum* (L.) and *V. album* (L.) were collected in Lagos, South Western, Nigeria. 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: The method of extraction of plant materials was as described by Wang and Weller[10]. The solid residues obtained after evaporation were dried, weighed as recommended by Patrick-Iwuanyanwu *et al.*[11] and reconstituted in 0.5% dimethyl sulfoxide (DMSO) (Sigma). They were 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) as recommended by Beltran[12].

Phytochemical screening: The extracts were reconstituted in methanol extraction solvent and then tested by standard phytochemical methods according to the methods of Evans[13] for the presence of alkaloids, cardiac glycosides, combined anthraquinones, flavonoids, free anthraquinones, saponins, tannins and terpenes.

HPLC analysis: Chromatographic analysis to characterize the plant extracts was performed by high performance liquid chromatography (HPLC) on the dried extracts using HPLC grade methanol (Surechem Products, UK) and HPLC grade water (Surechem Products, UK) at the ratio of 50:50. The mobile phase from the solvent bottles flows through the pump and the column (Agilent Technologies, 1120 Compact LC, Germany) to the detector which contains a flow cell to detect each separated compound band whose corresponding electrical signal known as a chromatogram was sent to a computer data station[14].

Evaluation of cellular toxicity: The method used was based on cellular morphologic changes as recommended by Park *et al.*[15]. Briefly Vero cells were prepared at a density of 8×10^4 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 24 h at 37°C. The positive control was Virest 200 mg, a brand of Acyclovir (HovidBhd, Malaysia). 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).

Virus propagaton: Measles (Edmonston-Zagreb strain, Serum Institute, Hyderabad, Pune, India), Polio (types 1, 2 and 3, Serum Institute, Hyderabad, Pune, India) and Yellow Fever (17D strain, FSUE of Chimakov IPVE, Russian Acad. Med. Sci.) viruses were isolated from thire respective vaccines obtained from Institute of Child Health, University College Hospital (UCH), Ibadan. Herpes simplex virus-1 (HSV-1) was isolated from the serum of a male patient who presented with HSV-1 symptoms at the Central Research Lab, Lagos University Teaching Hospital (LUTH) and confirmed to be HSV-1 using PCR. Using Reed-Muench method, viral infectivity ($1000\ TCID_{50}\ mL^{-1}$) was prepared from each of the stocks (MV $10^{-1.5}$, HSV-1 $10^{-3.5}$, YFV$10^{-4.5}$ and PV$10^{-4.5}$).

Virucidal activity: The virucidal activity was measured by *in vitro* incubation of viruses with the extracts. Briefly 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% MEM 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. Also added in triplicate were 100 µL of acyclovir positive control+100 µL 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[16]. The setup was monitored every day under the inverted microscope (Inverskop 40C) for 7 days and scored for the presence of virus-induced CPE[17].

Adsorption/Post adsorption assays: About 100 µL of Vero cell line was added to each of 96-well of a micro titer plate and incubated with 100 µL of different concentrations of each plant extract at 37°C for 2 h in a 5% CO_2. The extracts were removed and washed with PBS and cell monolayers were infected with 100 µL of 100 $TCID_{50}$ of each virus dilution. This was incubated for 7 days and the presentation of CPE was monitored using an inverted microscope (Inverskop 40C) and scored[18]. A Hundred microliter of 100 $TCID_{50}$ of each virus dilution in 1% MEM medium was added to Vero cell monolayer and incubated in a 5% CO_2 incubator for 2 h. Then viral inoculum was aspirated and discarded and cell monolayer washed with PBS, 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 4°C) and scored.

Statistical analysis: Cytotoxicity analyses of samples were performed in duplicates, antiviral studies were performed in triplicates. The IC_{50} values were calculated using GraphPad Prism software version 5.01 linear regressions (Graphpad Software Inc., U.S.A). For the susceptibility of Vero cell to the extracts/fractions and the test viruses to the extracts/fractions, comparisons were ascertained using a paired t-test. A $p<0.05$ at 95% confidence interval was considered significant.

RESULTS

Phytochemical analysis: The result in Table 1 summarized the qualitative phytochemical profile of *Bryophyllum pinnatum* (L.) and *Viscum album* (L.). It could be observed that both extracts contain all the phytochemicals tested, with phenolic compounds being more abundant in *V. album* (L.) than in *B. pinnatum* (L.). On the other hand, terpenoids and Steroids were more abundant in *B. pinnatum* (L.) than in *V. album* (L.).

Toxicity profile: The result in Fig. 1 showed the toxicity profile and IC_{50} of the methanol extracts of *B. pinnatum* (L.) and *V. album* (L.). The MNTC of BPM was 0.016 μg μL^{-1} with IC_{50} of 0.063 μg μL^{-1}, while that of VAM was 0.063 μg μL^{-1} with IC_{50} of 0.313 μg μL^{-1}. The MNTC of ACI was 0.063 μg μL^{-1} and IC_{50} of 4.56 μg μL^{-1}. The IC_{50} of all the extracts was found to be higher than their 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 none was observed on the wells containing cells and extracts only.

Virucidal activities: The result in Table 2 showed result of the virucidal analysis of the extracts of *B. pinnatum* (L.) and *V. album* (L.). It could be observed that only two of the viruses, Measles virus and Herpes simplex Virus-1, were susceptible to *V. album* and *B. pinnatum* (L.), respectively. Measles virus was susceptible to *V. album* at two MNTCs of 0.063 μg μL^{-1} and 0.031 μg μL^{-1}, while *B. pinnatum* (L.) inhibited Herpes Simplex Virus -1 at 0.016 μg μL^{-1}. Polio virus and yellow fever virus were resistant to both methanol extracts at all the concentrations tested. All the viruses were resistant to Acyclovir, the drug used as a positive control in this study.

Adsorption and post adsorption antiviral activities: The result as shown in Table 3 represented the results of the adsorption and post adsorption assays of *B. pinnatum* (L.) and *V. album* (L.) methanol extracts on the test viruses. While

Table 1: Phytochemical profile of *B. Pinnatum* and *Viscum album*

Constituents														
	Alkaloids		Cardiac glycosides		Anthraquinones		Flavonoids							
Extracts	Wanger's	Dragendorff's	Kedde's	Kellear Killani's	Free anth. Bontrager's	Combined Modified bontrager's	Ferric chloride	Lead acetate	Saponins	Tannins	Terpenes	Phenol	Steriod	
B. pinnatum	++	++	+	+	+	+	++	++	++	+	++	+	++	
V. album	++	++	+	+	+	+	++	++	+	+	+	+++	+	

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

Table 2: Virucidal activity of *B. pinnatum* and *V. album* methanol extracts (µg µL^{-1}) against 100 $TCID_{50}$ of the test viruses

	Extract concentration (µg µL^{-1})									
Extract	1	0.5	0.25	0.125	0.063	0.031	0.016	0.008	0.004	0.002
Measles										
BPM							1$^+$	4$^+$	4$^+$	
VAM					0	0	4$^+$			
ACI					4$^+$	4$^+$	4$^+$			
Ext cont					0	0	0			
Cell con					0	0	0			
Polio										
BPM							4$^+$	4$^+$	4$^+$	
VAM					4$^+$	4$^+$	4$^+$			
ACI					4$^+$	4$^+$	4$^+$			
Ext cont					0	0	0			
Cell con					0	0	0			
Yellow fever										
BPM							4$^+$	4$^+$	4$^+$	
VAM					4$^+$	4$^+$	4$^+$			
ACI					4$^+$	4$^+$	4$^+$			
Ext cont					0	0	0			
Cell con					0	0	0			
Herpes simplex virus										
BPM							0	2$^+$	3$^+$	
VAM					1$^+$	1$^+$	1$^+$			
ACI					4$^+$	4$^+$	4$^+$			
Ext cont					0	0	0			
Cell con					0	0	0			

4+: Complete (100%) cytopathic effect (CPE), 3$^+$: 75% CPE, 2$^+$: 50% CPE, 1$^+$: 25% CPE, 0 No CPE, Key: BPM: *B. pinnatum* Methanol, VAM: *V. album* Methanol, ACI: Acyclovir

Table 3: Pre and post-infection antiviral activity tests of methanol extract of *B. pinnatum* and *V. album* on 100 $TCID_{50}$ of measles and HS-1 viruses

	Extract concentration (µg µL^{-1})											
	Pre-infection antiviral activity						Post-infection antiviral activity					
Extracts	0.063	0.031	0.016	0.008	0.004	0.002	0.063	0.031	0.016	0.008	0.004	0.002
Measles virus												
BPM			4$^+$	4$^+$	4$^+$					4$^+$	4$^+$	4$^+$
VAM	4$^+$	4$^+$	4$^+$					0	0	4$^+$		
Ext con	0	0	0					0	0	0		
Cell con	0	0	0					0	0	0		
Herpes simplex virus-1												
BPM			0	0	4$^+$					4$^+$	4$^+$	4$^+$
VAM	4$^+$	4$^+$	4$^+$					0	4$^+$	4$^+$		
Ext con	0	0	0					0	0	0		
Cell con	0	0	0					0	0	0		

4$^+$: Complete (100%) cytopathic effect (CPE), 3$^+$: 75% CPE, 2$^+$: 50% CPE, 1$^+$: 25% CPE, 0 No CPE

B. pinnatum (L.) showed its activity against Herpes Simplex Virus-1 at the adsorption assay, *V. album* (L.) was active in post adsorption activity against MV and Herpes Simplex Virus-1.

Selectivity index: The IC_{50} and selectivity index of the extracts that inhibited the test viruses were represented in Table 4. It could be observed that the SI exceeded the IC_{50} of the extracts. This means that the extracts were all safe to be used directly as drugs or as pharmaceutical raw materials for drug production against the susceptible viruses.

Extracts characterization: The HPLC analysis of the crude methanol extract of *B. pinnatum* (L.) and *V. album* (L.), respectively were shown in Fig. 2 and 3. The presence of the chromatograms represented as spikes was for ease of

Table 4: About 50% inhibitory concentration (IC_{50}) and selectivity index (SI) of extracts that inhibited the test viruses

	IC_{50} (µg µL^{-1})		
Extracts	Vero cell	Virus replication	SI
Virucidal activity			
Measles virus			
VAM	0.313	0.031	10.1
Herpes simplex virus-1			
BPM	0.063	0.004	15.8
Pre-infection antiviral activity			
Herpes simplex virus-1			
BPM	0.063	0.004	15.8
Post-infection antiviral activity			
Measles virus			
VAM	0.313	0.004	8.03

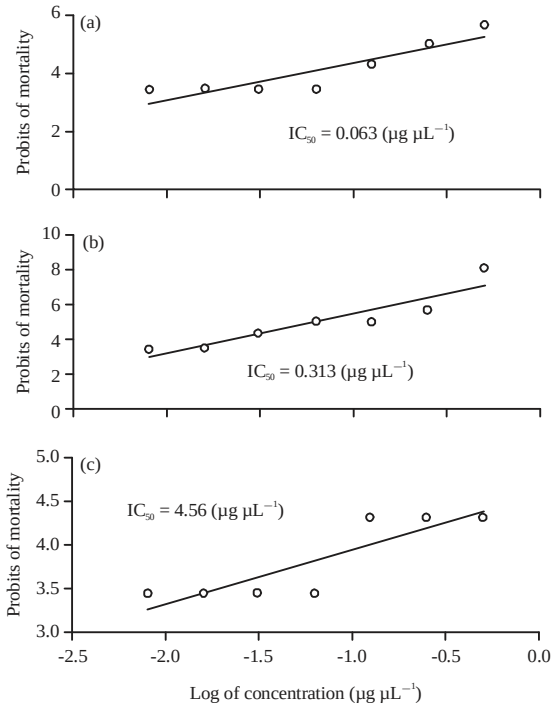

Fig. 1(a-c): Toxicity profile and IC_{50} of methanol extracts of (a) *Bryophyllum pinnatum*, (b) *Viscum album* and (c) Acyclovir

characterization of the compounds of both plants. The largest chromatogram of *B. pinnatum* (L.) had the retention time of 0.565 min and percentage peak area of 27.6%, while the largest in *V. album* (L.) had 2.266 min and percentage peak area of 58.4%.

DISCUSSION

In Nigeria, many indigenous plants are used in herbal medicine to cure diseases and heal injuries[17]. Such medicinal plants include *Bryophyllum pinnatum* (L.) and *Viscum album* (L.). This study has shown the toxicity profile of these two important medicinal plants and determined their maximum non-toxic concentration and IC_{50} necessary for antiviral therapeutics. However as the result showed in Fig. 1 *B. pinnatum* (L.) with IC_{50} of 0.063 µg µL^{-1} is considered more toxic than *V. album* with IC_{50} of 0.313 µg µL^{-1}. This toxicity study showed that although both plants are edible, they should be consumed with caution since it was observed that their methanol extracts could damage mammalian cells. The observation made in this study was confirmed by the study of Reppas[19], who reported that two adult cattle died of myocardial degeneration within 48 h of being fed a large amount of *B. pinnatum* (L.), probably due to the presence cardiac glycosides and bufadienolide in the plant tissues. Similarly the fruits of most *V. album* (L.) species were described as toxic berries which could lead to vomiting, hypotension, cerebral dysfunctions and death by a heart attack if ingested in large numbers[20]. However, the toxicity profile of both plants on Vero cell lines was a pointer to the fact that they could possess strong anti-cancer properties as a result of their flavonoid constituents.

As a rich source of phytochemical (Table 1), *B. pinnatum* (L.) and *V. album* (L.) could become potential sources of useful raw materials for drugs production. Figure 2 and 3 also represent the abundance of phytochemicals in these two plants as shown by their chromatograms which have percentage peak areas of 58.4% for *V. album* (L.) and 27.6% for *B. pinnatum* (L.). These rich bioactive molecules have potentials to be used in treatment of various ailments. For instance, phenolic compounds inactivate micro-organisms by enzyme inhibition[21], binding of polyphenols to the protein coat of viruses or binding to the virus and/or the protein of the host cell membrane thus arresting adsorption of the virus[22]. Another constituent, flavonoids are phytochemicals universally known to be a potent and specific inhibitor of the most frequent causative agents of common cold (the

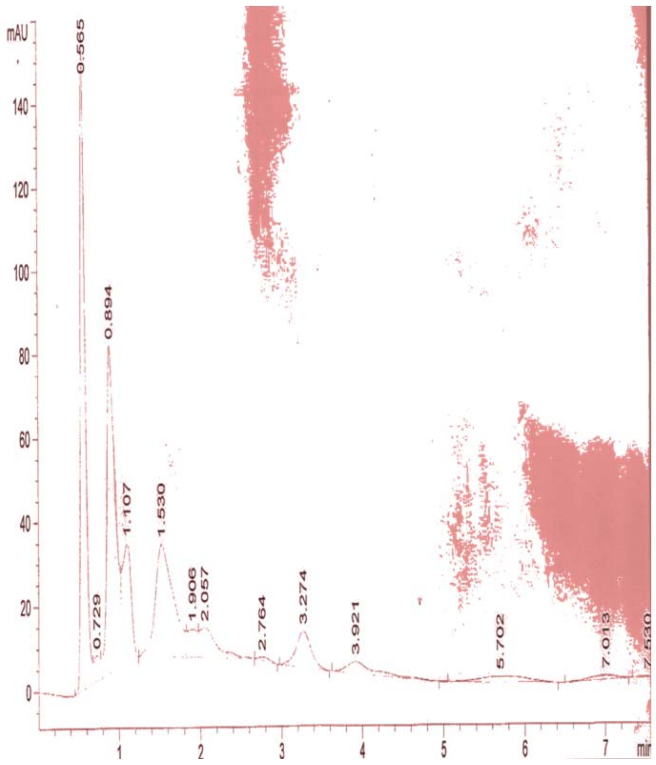

Fig. 2: Chromatogram of *B. pinnatum* (L.)

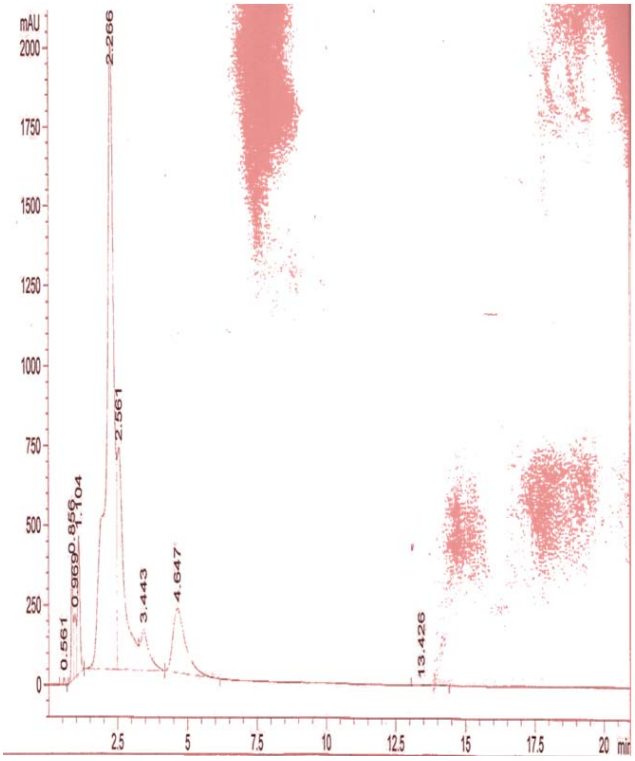

Fig. 3: Chromatogram of *V. album* (L.)

picornaviruses and Rhinoviruses)[23] and exhibit various antiviral effects against a number of viruses. Terpenes are active against bacteria, fungi, viruses and protozoa[24]. The presence of these phytochemicals may have contributed to the antiviral activities of the methanol extracts of both plants observed in this study.

The results of the virucidal activities of both plants revealed their strong potential as antiviral agents. The fact that the crude extracts of *B. pinnatum* (L.) and *V. album* (L.) were able to inhibit HSV-1, an enveloped DNA virus and MV an enveloped RNA virus, respectively, could mean that the plants were broad spectrum in action (Table 2). The observed antiviral activities could be due to the presence of bufadienolides, which have been reported to be the active components of *B. pinnatum* (L.) responsible for their anti-bacterial, anti-tumor, anti-viral, anti-fungal, anti-hypertensive, diuretic, anti-asthmatic, anti-histamine, cancer preventive and insecticidal properties. However, since polio, the only non-enveloped virus used in this study was observed to be resistant, this could only mean that enveloped viruses were more susceptible to the extracts of *B. pinnatum* (L.) and *V. album* (L.) than the non-enveloped viruses[25]. Yellow fever virus was also resistant to both extracts. Extensive studies have also been done on extracts from the leaves of *V. album* (L.) and it has variously been reported that the plant was strongly antiviral in action[26]. Evidences abounds also about the effectiveness of mistletoe-lectins obtained from *V. album* (L.) for the clinical treatment of cancer, rheumatoid arthritis, immune diseases and HIV/AIDS[27].

Having inhibited both HSV-1 at the pre-infection stage of this study, *B. pinnatum* (L.) could become a veritable source of pharmaceutical raw material for drug production against viral entry/attachment, meaning that it could become a potential preventive therapy for this susceptible virus. On the other hand *V. album* (L.) could only inhibit MV and HSV-1 in the post-infection antiviral assay and as a result, it could become a potent treatment modality for infection due to this virus as shown in Table 3.

The *B. pinnatum* (L.) and *V. album* (L.) displayed a high level of safety index (SI) as shown in Table 4. With an SI which exceeded the IC_{50} of the methanol extracts of the two plants, the safety of *B. pinnatum* (L.) and *V. album* (L.) as useful pharmaceutical raw materials for drugs production against the susceptible viruses could be said to have been established. According to De Clercq, (28), a drug or extract could be considered safe for use if the SI of such substances exceeded one.

CONCLUSION

This study has shown that, *Bryophyllum pinnatum* (L.) (Odaaopua) and *Viscum album* (L.) (Awuruse), which inhibited measles and HSV-1 and are freely available and abundant in nature in Nigeria, could be harnessed and processed into drugs that could be used as broad spectrum antiviral agents for the control of the susceptible viruses.

SIGNIFICANCE STATEMENT

This study discovered the antiviral potentials of two locally available herbs in Nigeria that can be beneficial for the prevention and treatment of viral infections of public health importance. This study will help the researchers to uncover the critical areas of viral drug development that many researchers were not able to explore. Thus a new theory on antiviral research may be arrived at.

REFERENCES

1. Huang, J., D. Su, Y. Feng, K. Liu and Y. Song, 2014. Antiviral herbs-present and future. Infect. Disord.-Drug Targets, 14: 61-73.
2. Kitazato, K., Y. Wang and N. Kobayashi, 2007. Viral infectious disease and natural products with antiviral activity. Drug Discov. Ther., 1: 14-22.
3. Iwu, M.M., 1993. Handbook of African Medicinal Plants. CRC Press, Boca Raton, ISBN-13: 9781466571976, pp: 20-22.
4. Tatsimo, S.J.N., J. de Dieu Tamokou, L. Havyarimana, D. Csupor and P. Forgo *et al*., 2012. Antimicrobial and antioxidant activity of kaempferol rhamnoside derivatives from *Bryophyllum pinnatum*. BMC Res. Notes, Vo. 5. 10.1186/1756-0500-5-158.
5. Kamboj, A. and A.K. Saluja, 2009. *Bryophyllum pinnatum* (Lam.) Kurz.: Phytochemical and pharmacological profile: A review. Pharmacogn. Rev., 3: 364-374.
6. Zuber, D., 2004. Biological flora of Central Europe: *Viscum album* L. Flora-Morphol. Distrib. Funct. Ecol. Plants, 199: 181-203.
7. Watson, D.M., 2001. Mistletoe-A keystone resource in forests and woodlands worldwide. Annu. Rev. Ecol. Systemat., 32: 219-249.
8. Bussing, A. and M. Sheitzel, 1999. Apoptosis-inducing properties of *Viscum album* L. extracts from different host trees, correlate with their content of toxic mistletoe lectins. Anticancer Res., 19: 23-28.
9. Davies, J., 2003. Anti-viral activity of plant extracts and herbs. Int. J. Trop. Med., 2: 49-54.
10. Wang, L. and C.L. Weller, 2006. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Technol., 17: 300-312.

11. Patrick-Iwuanyanwu, K.C., N. Onyeike Eugene 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 Pharm. Sin., 2: 9-16.
12. Beltran, O., 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://search.proquest.com/docview/304372126
13. Evans, W.C., 2009. Trease and Evans Pharmacognosy. 15th Edn., Sunders Elsevier, London, UK., ISBN: 9788 131200872, pp: 173-415.
14. Sasidharan, S., Y. Chen, D. Saravanan, K.M. Sundram and L. Yoga Latha, 2011. Extraction, isolation and characterization of bioactive compounds from plant's extracts. Afr. J. Tradit. Complement. Altern. Med., 8: 1-10.
15. 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., Vol. 9. 10.1186/1472-6882-9-29.
16. 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. Plants Res., 3: 771-775.
17. Barakat, M.N., R.S. Abdel Fattah, M. Badr and M.G. El-Torky 2010. *In vitro* culture and plant regeneration derived from ray florets of *Chrysanthemum morifolium*. Afr. J. Biotechnol., 9: 1151-1158.
18. 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.
19. Reppas, G.P., 1995. *Bryophyllum pinnatum* poisoning of cattle. Aust. J. Vet., 72: 425-427.
20. Didier, D.S., E.O.N. Laurier, N. Din, P.R. Jules and T. Victor *et al*., 2009. An assessment on the uses of Loranthaceae in ethno pharmacology in Cameroon: A case study made in Logbessou, North of Douala. J. Med. Plants Res., 3: 592-595.
21. Mason, T.L. and B.P. Wasserman, 1987. Inactivation of red beet β-glucan synthase by native and oxidized phenolic compounds. Phytochemistry, 26: 2197-2202.
22. Vanden Berghe, D.A., A.J. Vlietinck and L. van Hoof, 1986. Plant products as potential antiviral agents. Bull. Inst. Pasteur, 84: 101-147.
23. Semple, S.J., S.M. Pyke, G.D. Reynolds and R.L.P. Flower, 2001. *In vitro* antiviral activity of the anthraquinone chrysophanic acid against poliovirus. Antiviral Res., 49: 169-178.
24. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.
25. Igwe, S.A. and D.N. Akunyili, 2005. Analgesic effects of aqueous extracts of the leaves of *Bryophyllum pinnatum*. J. Pharm. Biol., 43: 658-661.
26. Karagoz, A., E. Onay, N. Arda and A. Kuru, 2003. Antiviral potency of mistletoe (*Viscum album* ssp. album) extracts against human parain uenza virus type 2 in Vero cells. Phytother. Res., 17: 560-562.
27. Boghaei D.M. and Z. Javani, 2010. The anti-HIV herbaceou drug from *Viscum album*: Theoretical evaluation. J. Nano Chem. Agric., 1: 35-44.