Antiviral Potentials of *Enantia chlorantha* Extracts on Yellow Fever Virus

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**Abstract:** *Enantia chlorantha* plant used by traditional medical practitioners or in phytomedical practice in the treatment of diseases such as fevers and influenza were investigated for their inhibitory activities on the yellow fever virus in the tissue cell culture using Vero cells. The dried powdered stem bark of *E. chlorantha* was extracted with water and the extracts evaporated to dryness. Dry residue were dissolved in respective solvents (1:10 v/v) and tested for antiviral activity at P < 0.01 against yellow fever virus by standard laboratory procedures. The water extract of the plants assayed for cytotoxicity in Vero cells showed that the Minimum Inhibitory Concentrations (MICs) of *E. chlorantha* was 0.025 mg/ml. each of the extracts was used at the established MICs. These extracts were mixed with equal volumes of 100TCID50 Yellow Fever Virus (YFV) in confluent monolayer of Vero cells. The extract showed antiviral activities against yellow fever virus. *Enantia chlorantha* resulted in inhibition of yellow fever viruses at MICs of 0.025 mg/ml. *E. chlorantha* at 0.025 mg/ml ceased to be cytopathic to the cell line. The result of the study revealed that the water extract of *E. chlorantha* showed significant antiviral activity. Based on this experimental evidence, the extracts were considered effective against YFV as they completely inhibited the infectivity of YFV as evident in complete absence of Cytopathic effects (CPEs). It should therefore be recommended that application of extracts from *E. chlorantha* could help in the treatment of yellow fever infections. It is possible that more potent components especially against YFV might reside in the polar fractions which should form the focus of future investigation.


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**Key words:** Antiviral activity, cytotoxicity, cytopathic effects, *Enantia chlorantha*, yellow fever virus

1. Introduction

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being (Igbinosa et al., 2009). Their role is two fold in the development of new drugs: (1) they may become the base for the development of a medicine, a natural blueprint for the development of new drugs or; (2) a phytomedicine to be used for the treatment of diseases (Igbinosa et al., 2009). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world’s population, especially in the developing world (Igbinosa et al., 2009). Higher plants have been shown to be a potential source for the new antimicrobial agents (Alim et al., 2009). The screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases. A number of reports concerning the antimicrobial screening of plant extracts of medicinal plants have appeared in the literature, but the vast majority has not been adequately evaluated (Alim et al., 2009).

In Africa, phytomedical preparations have been used over a long period for the treatment of ailments (Udobi and Onaolapo, 2009). This is because western medicine is not available in some places due to a wide range of reasons; among which include that the first line drugs have become ineffective due to resistance. Herbal preparations are becoming more widely used by people all over the world as they understand their availability and affordability. Apart from these, the gentle strength in herbs and the fact that most of them can be used safely without a known side effect makes it more popular. Workers in the field of plant medicinal research regarded higher plants as living chemical factories providing a vast number of chemical substances that display a variety of biological actions (Udobi and Onaolapo, 2009).

The more current and most effective antibiotics are very expensive and out of reach of many Africans, majority of whom reside in the rural areas. These antibiotics are also associated with some serious side-effects. A medicinal plant, such as *E. chlorantha*, is readily available and affordable (Adesokan et al., 2007). *Enantia chlorantha* Oliv. (African yellow wood) is a medicinal plant that has been used traditionally in southwestern, Nigeria. It belongs to the family Annonaceae and has diverse uses. One of those medicinal plants that have been used in many localities in Nigeria to manage fever is *Enantia chlorantha* (Adesokan et al., 2008). *E. chlorantha* Oliv (family-Annonaceae) locally known as Awogba, Oso pupa or Dokita igbo (Yoruba), Osomolu (Ikale), Kakerim (Boki) and Erenba-vbogo (Bini), is widely distributed along the coasts of West and Central Africa. It is also very common in the forest regions of Nigeria (Adesokan et al., 2008). It is an ornamental tree which may grow up to 30 m high, with dense foliage and spreading crown. The outer bark which is thin and dark brown is fissured geometrically while the inner bark is brown above and pale cream beneath. The stem is fluted and aromatic while the elliptic leaves are about...
0.14 – 0.15 m long and 0.05 – 0.14 m broad (Adesokan et al., 2008).

In an attempt to establish the components responsible for the use of Enantia chlorantha against cutaneous leishmaniasis in local traditional medicine, a well-known palmatine was isolated by Nkwengoua et al. (2009) in substantial amounts from a methanolic bark extract of this plant species. Palmatine therein obtained exhibited a significant inhibitory activity on growth of both Trypanosoma cruzi and Leishmania infantum (Nkwengoua et al., 2009). Several studies have shown that the stem bark of E. chlorantha possess wide spectrum antimicrobial activity including against Klebsiella aeruginosa (Adesokan et al., 2007) and brewer’s yeast induced pyrosis in rats (Adesokan et al., 2008).

Yellow fever virus is the causative agents of yellow fever disease. Yellow fever is one of the viral haemorrhagic fevers, with classical features of hepatitis, which is the reason for the yellow colouration of the skin (jaundice) and the name yellow fever of the disease. Chemotherapeutic agents or the use of antiviral therapy is to inhibit viral replication in the host cells. Antiviral agent is that which can produce either protective advantage of the virus infected host of any material and can significantly enhance antibody formation and improve antibody activities. One approach that has been used for the discovery of antimicrobial agents from natural sources is based on the evaluation of traditional plant extracts (Uysal-Gökçe et al., 2004; Özçelik et al., 2005; 2006; 2008, 2009, Koca et al., 2009; Orhan et al., 2009; Kan et al., 2009; Jyoti & Verma, 2011; Abeer and Afaf, 2011).

The term ‘antiviral agents’ has been defined in very broad terms as ‘substances other than a virus or virus containing vaccine or specific antibody which can produce either a protective or therapeutic effect to the clear detectable advantage of the virus infected host (Abonyi et al., 2009). Many workers have extensively investigated antiviral and chemotherapeutic effect of different chemicals and extracts of plants or biological bye products. Behbahani (2009) reported the anti-viral activity of the methanolic leaf extract of an Iranian medicinal plant “Hyssopus officinalis” against HSV. Ojo et al. (2009) reported on antiviral properties of two Nigerian plants on measles, yellow fever and polio viruses. Kan et al. (2009) reported the in vitro antiviral activities under cytotoxic doses against HSV-1 and parainfluenza-3 viruses of Cicer arietinum L. (Chickpea), Madagada and Tewtrakul (2010) reported anti-HIV-1 protease activity screening of some Tanzanian medicinal plants of the genus Garcinia (Clusiaceae). Few Garcinia species reported in the literatures have been investigated for their HIV-1 protease (PR) inhibitory effects (Magadada and Tewtrakul, 2010). Okoro et al. (2010) screened polyphenols from the ethnomedicinal plants (Momordica charantia, Senna alata and Nauclea laffifolia) for their antioxidant and antimicrobial potentials and activities against pathogenic microorganisms (Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli and Candida albicans). In another study, Cassia occidentalis and C. zambensis exhibited active antimicrobial activity against the enterobacteria tested that is, S. typhi and E. coli than N. leavis and the combination of the three plant sources (Ajayi and Akinlola, 2010). Ricinus communis was used orally for body ache, maintained good health and for treating loss of hair (Harun-or-Rashid et al., 2010). Tessong and Obi (2006) and Bessong et al. (2005, 2006) also reported the inhibitory activities of Ricinus communis against HIV-1.

Two major obstacles to the development and use of effective antiviral chemotherapy are the close relationship that exists between the multiplying virus and the host cell, and that viral diseases can only be diagnosed and recognized after it is too late for effective treatment (Abonyi et al., 2009). In the first case, an effective antiviral agent must prevent completion of the viral growth cycle in the infected cell without being toxic to the surrounding normal cells. One encouraging development is the discovery that some virus specific enzymes are elaborated during multiplication of the virus particles and this may be a point of attack by a specific inhibitor (Abonyi et al., 2009). However, recognition of the disease state too late for effective treatment would render that antiviral agent useless even if they were available. Until early recognition of the disease state is provided, most antiviral chemotherapeutic agents will have their value as prophylactic agents (Abonyi et al., 2009).

The reason for the apparent lack of progress in antiviral chemotherapy as compared with the field of antibacterials has been a problem of selectivity (Abonyi et al., 2009). Any antiviral agent must selectively kill the pathogenic organism in the presence of other living cells (Abonyi et al., 2009). Sufficient biochemical differences exist between the metabolism of prokaryotic bacterial cells and mammalian cells to enable selectivity to be achieved, hence the early development of antibacterial agents, which were safe for human use (Abonyi et al., 2009). Viruses on the other hand, despite their apparent simplicity present a bigger problem. This is because during their replicative cycle, they become physically and functionally incorporated into the host cells and it is therefore difficult to distinguish unique biochemical features suitable for selective attack. Some viruses also persist in a latent infection, in which case, antiviral drugs are less likely to be effective (Abonyi et al., 2009). However increased understanding of the molecular events of virus infections has meant that the search for antiviral drugs against specific targets can be conducted on a more rational basis (Abonyi et al., 2009).

This current study was designed to evaluate the antiviral effects of bark extract of E. chlorantha on Yellow Fever Virus (YFV) in vitro using Vero cell line, to determine the minimum tolerance dose of these plants extracts on Vero cells, and to justify in this regard, the administration of these medicinal plants by the traditional medicine practitioners.

2. Materials and Methods

2.1. Collection and Identification of Plant Materials
The plant bark of *E. chlorantha* was purchased at Bodija market and identification was done at the Forestry Research Institute of Nigeria by Mr T.K. Odewo.

2.2. Preparation of Extracts

Extraction of the *E. chlorantha* barks was carried out in the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria. The method described by Yakubu et al. (2005) was employed. Briefly, the plant stem bark was cut into pieces with the aid of a sterile knife and oven-dried at 40°C until constant weight was obtained. The dried pieces were then pulverized with an electric blender (Blender/Miller III, model MS-223, China). The powdered material was stocked in a plastic container from which 20 g each was separately percolated in 100 ml of cold distilled water and absolute ethanol for 48 h at room temperature with constant shaking. The extract was then filtered with Whatman No. 1 filter paper and the resulting filtrate for the water extract was concentrated on a steam bath to give 5.00 g of the residue (% yield of 25.00 g ± 0.05). The residues were reconstituted in distilled water to give the required doses of 25, 50, 100 and 200 mg/kg body weight of the aqueous extracts. The extracts were concentrated, after complete solvent evaporation, spurred each of these solvent extract was weighed and preserved at 5°C in an airtight bottle until further use. One gram of water extract was dissolved in 10ml of water which served as a test extract for 5.00 g of the residue (% yield of 25.00 g ± 0.05). The residues were reconstituted in distilled water to give the required doses of 25, 50, 100 and 200 mg/kg body weight of the aqueous extracts. The extracts were concentrated, after complete solvent evaporation, spurred each of these solvent extract was weighed and preserved at 5°C in an airtight bottle until further use. One gram of water extract was dissolved in 10ml of water which served as a test extract for antiviral activity assay (Kumaraswamy et al. 2008; Adebayo and Ishola 2009).

2.3. Cells and Media

African green monkey kidney (Vero) cells were passaged in Minimum Essential Eagle Medium (MEM) (Gibco BRL, Scotland, UK), supplemented with 5% Fetal Calf Serum (FCS) (BioWhittaker Europe, Germany and antibiotics; cell cultures were cultivated at 37°C in the presence of 5% CO₂ till the formation of confluent monolayers. In the antiviral experiments 0.5% FCS was added.

2.4. Viral Propagation

The hyperattenuated vaccine strain of Yellow Fever Virus (YFV) from Dakar, Ghana was grown in mouse brain of 2 day old mice at the Department of Virology, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria. The YFV harvested from the mice were grown in Vero cells in the presence of 2 μg/ml trypsin (Sigma); the infectious titre was 10⁵-7 - 10⁷ TCID50/ml (50% tissue culture infectious doses/ml). The virus stocks were stored at -80°C.

2.5. Antiviral Assays

The technique described is a modified form of procedures previously described in detail (Ojo et al., 2009). Vero cells were grown in Dulbecco’s Modified Eagle Medium (MEM) containing 5% fetal bovine serum (all cell culture reagents were obtained from GIBCO Life Sciences, Ontario) in 96-well microtest trays (Falcon), 0.2 ml per well. When the cells formed confluent monolayers, they were used for the assays. Each plant extract was diluted 1:100 in MEM plus 0.1% serum and filtered through a sterile syringe filter 0.2 μ pore diameter. The filter equivalent to 1,000 μg/ml dried plant material and 1% of the 70% ethanol was the starting test material. In the standard procedure, serial 2-fold dilution of the extract were made (in duplicate) in MEM plus 0.1% serum across a row of wells in an empty 96-well microtest tray. With the aid of a multipipettor, these diluted extracts were transformed to the aspirated Vero cell monolayers of another 96-well tray, 0.1 ml per well. The cultures were incubated at 37°C for 60 min and examined microscopically for possible immediate cytoxic effects. Then 0.1ml of virus (yellow fever virus), comprising 100 pfu in MEM + 0.1% serum was added to each well. Controls induced cells with no virus and cells infected with untreated virus. Cultures were inspected periodically in the microscope for viral CPE.

2.6. Cytotoxicity Assays

The procedure was similar to the antiviral assay except that no virus was added to the wells and following light exposure, the trays were returned to the incubator for periodic microscopic assessment of changes in cell morphology or visible toxic effect (Ojo et al., 2009). The cells grown in the absence of the extracts were used as 100% cell survival. The concentration at which the cell number were reduced to 50% of that when compared with the cell controls was taken as 50% cytotoxic dose (CD50). The concentration which had no effect on the cell number (maximum tolerated concentration or minimum cytotoxic dose (MCD)) was also observed.

3.0. Result Analysis

3.1. Cytotoxicity assays

The screening of the crude water extracts of plants were tested for maximum tolerance dose on Vero cells using different concentration. The results of the cytotoxicity assays are shown in Table 1. The water extracts of *E. chlorantha* were found to have maximum tolerance (MTD) or minimum cytotoxic dose (MCD) at 0.025mg/ml on Vero cells. *Enantia chlorantha* demonstrated a lower level of toxic effect on the cell line as shown in Table 1. The cytotoxicity assays was used in this study. This protocol involving continuous exposure of the cells to extract for 5 days, permits detection of cytotoxic effects leading to cell death as well as more subtle effects on the cells that may not be deleterious e.g. alteration of cell shape to a more rounded morphology (Ojo et al., 2009). The extracts produced such changes in cell morphology at concentration higher than 0.025 mg/ml. The effectiveness of the *E. chlorantha* against the yellow has been observed. Extract of *E. chlorantha* was however inhibitory on yellow fever viruses. Yellow fever virus was inhibited at a minimum concentration of 0.025mg/ml. This is in line with other scientists who established that crude
extracts of some plants and some pure compounds from such plants can potentiate the activity of antibiotics in-vitro (Udobi and Onaolapo, 2009; Ojo et al., 2009). The cytotoxicity assay of the extract on Vero cells showed that E. chlorantha is less toxic with minimum concentration of 0.025mg/ml. This shows that generally the phytochemical constituents in E. chlorantha is less toxic to Vero cells. This justifies the raw application of the crude water extracts of the plant when taken orally or applied directly on the body.

Table 1: Cytotoxicity assays of Enantia chlorantha

<table>
<thead>
<tr>
<th>Enantia chlorantha extract Concentration (mg/ml)</th>
<th>Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>+</td>
</tr>
<tr>
<td>2.5</td>
<td>+</td>
</tr>
<tr>
<td>0.25</td>
<td>+</td>
</tr>
<tr>
<td>0.025</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: = absence of cytotoxicity  
+ = presence of cytotoxicity

3.2. Virus propagation

Tables 2a and 2b show the propagation of yellow fever virus (YFV) in two sets of mice. The YFV grows readily in the brain of 1-2 day-old mice. The results showed that at day 1, 2, and 3, the mice showed no visible signs and symptoms of the YFV inoculated intracerebrally at day 0. At day 4, two of the mice showed signs of sickness which included sluggishness, crouching distinctively in one corner by the mice involved. At day 5, two of the mice became visibly sick but were not dead yet. At day 6, the two sick mice were eaten by their mothers and all the others were visibly sick (Table 2a and 2b).

3.3. Antiviral activities

Antiviral activities were measured as complete or partial inhibition of viral CPE (cytopathic effects) at concentrations of 25mg/ml, 2.5mg/ml, 0.25mg/ml, and 0.025mg/ml (Table 3). This study has shown the antiviral effect of water extract from stem bark of E. chlorantha. Bark extract of E. chlorantha was active against the YFV. It inhibited the virus 100% at minimum inhibitory concentration (MIC) of 0.025mg/ml. In vitro evaluation of the antimicrobial activities of bark of E. chlorantha has been previously reported by Adesokan et al. (2007). A bioactivity-directed fractionation of the root and stem-barks of E. chlorantha resulted in the isolation of palmatine chloride 1 and jatrohizine chloride 2 as the major antimicrobial constituents. MIC determinations indicated that these compounds were superior to those of well established broad spectrum antibiotics against some of the organisms used (Moody et al., 1995).

Table 2a: Virus propagation (1st passage)

<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>Period (st passage)</th>
<th>No. of mice inoculated</th>
<th>No. showing sickness (%)</th>
<th>No. confirmed sick</th>
<th>No. missing/lost (%)</th>
<th>No. eating (%)</th>
<th>No. dead</th>
<th>No. survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12</td>
<td>0/12</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>12(100.0)</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>0/12</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>12(100.0)</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>0/12</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>12(100.0)</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>0/12</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>12(100.0)</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>0/12</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>12(100.0)</td>
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<tr>
<td>5</td>
<td>12</td>
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</tr>
<tr>
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<td>0/12</td>
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<td>0(0.0)</td>
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<td>0(0.0)</td>
<td>0(0.0)</td>
<td>12(100.0)</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>0/12</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>12(100.0)</td>
</tr>
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</table>

Table 2b: Virus propagation (2nd passage)

<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>Period (st passage)</th>
<th>No. of mice inoculated</th>
<th>No. showing sickness (%)</th>
<th>No. confirmed sick</th>
<th>No. missing/lost (%)</th>
<th>No. eating (%)</th>
<th>No. dead</th>
<th>No. survived</th>
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<tr>
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<tr>
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<td>0(0.0)</td>
<td>12(100.0)</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>0/12</td>
<td>0(0.0)</td>
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<td>0(0.0)</td>
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<td>0(0.0)</td>
<td>12(100.0)</td>
</tr>
<tr>
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<td>12</td>
<td>0/12</td>
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<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>12(100.0)</td>
</tr>
<tr>
<td>Total</td>
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<td>0/12</td>
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<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>12(100.0)</td>
</tr>
</tbody>
</table>

Table 3: Antiviral activities of the Enantia chlorantha extract on yellow fever virus

<table>
<thead>
<tr>
<th>Well</th>
<th>Log of virus dilution</th>
<th>EXTRACTS CONCENTRATION (mg/ml)</th>
<th>CPE</th>
<th>NO CPE</th>
<th>CPE</th>
<th>NO CPE</th>
<th>Cumulative values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10^1(++)</td>
<td>0.250(1)</td>
<td>4</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>4/4 100</td>
</tr>
<tr>
<td>2</td>
<td>10^1(++)</td>
<td>0.133(2)</td>
<td>4</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>4/4 100</td>
</tr>
<tr>
<td>3</td>
<td>10^1(++)</td>
<td>0.025(3)</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4/4 100</td>
</tr>
<tr>
<td>4</td>
<td>10^1(++)</td>
<td>0.013(4)</td>
<td>4</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>4/4 100</td>
</tr>
<tr>
<td>5</td>
<td>10^1(++)</td>
<td>0.008(5)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2/4 50</td>
</tr>
<tr>
<td>6</td>
<td>10^1(++)</td>
<td>0.003(6)</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>16</td>
<td>0/4 0</td>
</tr>
<tr>
<td>7</td>
<td>CC(1)</td>
<td>CC(1)</td>
<td>4</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>4/4 100</td>
</tr>
<tr>
<td>8</td>
<td>VCC(1)</td>
<td>VCC(1)</td>
<td>4</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>4/4 100</td>
</tr>
</tbody>
</table>

Key:  
CPE = Cytopathic Effect of the virus on Vero cells  
+++ = Complete cells CPE  
++ = 75% CPE  
+ = partial inhibition of viral CPE (incomplete CPE)  
- = Inhibition of viral CPE  
CC = Cell control containing cell lines in maintenance medium  
EC = Extract control at MCD50  
VC = Virus control
Water extract of *E. chlorantha* bark completely inhibited the infectivity of the YFV against the Vero cell at concentration 0.025mg/ml of 100TCID$_{50}$ of the virus. The antiviral potential of the plant samples was screened in 2 model systems: reproduction of 2 vaccine strains of YFV in mice brain and of 2 YFV in Vero cells. The water extract of *E. chlorantha* showed good anti-YFV effects; the growth of YFV were reduced significantly (Table 3).

In this present study, bark of *E. chlorantha* showed antiviral effect against the YFV. It may be that the extracts of the *E. chlorantha* bark equally contains such alkaloid or active ingredient that can inhibit viral infectivity. *E. chlorantha* bark extract was active against the YFV. YFV is inactivated by treatment with extracts of this plant and it results in a 96-100% protection rate. The plant extracts were also been found to have anti-YFV action in infected Vero cells and in infected mice on both oral and subcutaneous administration. Another study however reported it to be more effective against dermotropic than keratogenic strains of the HSV (Vichikonova and Gorunyuova, 1972). Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being (Igbinosa et al., 2009). Their role is two fold in the development of new drugs: (1) they may become the base for the development of a medicine, a natural blue print for the development of new drugs or; (2) a phytomedicine to be used for the treatment of diseases (Igbinosa et al., 2009). Traditional medicine using plant extract continues to provide health coverage for over 80% of the world’s population, especially in the developing world (WHO 2002; Igbinosa et al., 2009). Higher plants have been shown to be a potential source for the new antimicrobial agents (Ali et al., 2009). The screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases. A number of reports concerning the antibacterial screening of plant extracts of medicinal plants have appeared in the literature, but the vast majority has not been adequately evaluated (Ali et al., 2009).

4.0. Conclusion

Plant tissue cultures have in recent times been found to have serious antiviral properties. More recently, cell cultures developed by biotechnological techniques have also been found to possess antiviral activities (Ibezim, 2003; Abonyi et al., 2009). For long, plants used as whole plants, tissue cultures or cell cultures have provided sources of antiviral agents (Abonyi et al., 2009). Other than plants, algae and lichens have also been employed as antiviral agents (Abonyi et al., 2009). Methods employed in screening plants and their extracts for antiviral activities include use of animal models, animal protection studies, egg inoculation studies and cell culture methods (Abonyi et al., 2009). Plants are able to produce compounds which though have no apparent function in the primary metabolism of the plant, have good activity against bacterial, fungal and some viral pathogens when they are able to find their way into and accumulate in them. These compounds have had an extensive history of use as therapeutic agents (Udobi and Onaolapo, 2009). In the course of our search for the antiviral properties of *E. chlorantha* extracts having various polarities, remarkable antiviral activities were determined against the YFV.

These extracts exhibited anti-YFV activity; evident by the complete absence of CPE when compared with the cell control. It may be that these extracts acted directly on the virus or coated the surface of the cell thereby preventing penetration and/or inhibiting the stages of viral replication. There is therefore, the need for further studies to elucidate and determine the active ingredients, pharmacological properties, mode of actions, chemospects and other therapeutic properties or values of these plants as antiviral agents. To this effect, there must be cooperation between the various modern health workers, researchers, scientists and the traditional system. Plants produce many organic substances which have value in the treatment of viral infection. It is essential to establish these active substances which act on the viral infections and replication, so that they can be used more effectively in treatment and prevention of viral diseases.

In conclusion, water extract of *E. chlorantha* bark have broad-spectrum antimicrobial activities with yellow fever virus investigated showing more susceptibility to the water plant extract of *E. chlorantha* stem bark. The broad-spectrum antiviral activities of the plant extract, possibly due to the identified alkaloids, further confirm its use as antiviral agent in folklore medicine of Nigeria and may thus be useful in the treatment of viral infections. The outcome of the antiviral screenings of *E. chlorantha* was impressive as the extracts possess activity against the yellow fever virus which was tested. From the findings of this study, it was confirmed that *E. chlorantha* extracts, have shown promising but differential *in-vitro* antiviral activity. It should therefore be recommended that application of extracts from these plants could help in the treatment of yellow fever infections. It is possible that more potent components especially against YFV could form the focus of future investigations.

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