**ABSTRACT**

**Aim:** The human immunodeficiency virus (HIV) remains a major public health concern despite the discovery and development of Highly Active Antiretroviral Therapies (HAART). There is as such a need to continue to search for new and effective therapies for this global pandemic. In an effort to discover new anti HIV agents, the aim of this study was to determine the anti HIV-1 activity of *Petiveria alliacea* and its metabolites.

**Methodology:** The extracts of *P. alliacea* and dibenzyl trisulfide were screened for anti HIV-1 properties in primary peripheral blood mononuclear cells (PBMCs) infected with the HIV-1JR-CSF strain.
**Results:** The anticancer metabolite of *P. alliacea* called dibenzyl trisulfide and the crude methanol and ethyl acetate extracts inhibited HIV-1 reverse transcriptase in infected cells, with EC_{50} concentrations of 5.60 µg/ml, 21.6 µg/ml and 68.0 µg/ml, respectively. The reference compound AZT had an EC_{50} value of 0.005 µg/ml. The tested extracts had IC_{50}/EC_{50} selectivity index (SI) values of ≥ 1.47. The results were confirmed in another assay measuring the expression of p24.

**Conclusion:** The results of this study indicate that extracts of *P. alliacea* may contain anti-HIV-1 metabolites that could provide leads for the discovery of new agents against the HIV virus.

**Keywords:** Anti HIV-1; Petiveria alliacea; Guinea hen weed; dibenzyl trisulfide; Jamaica; medicinal plant.

**1. INTRODUCTION**

The Human Immunodeficiency Virus (HIV) is the causative agent of the Acquired Immunodeficiency Syndrome (AIDS) which is considered one of the most important global health problems of the 21st century. Despite the discovery and development of Highly Active Antiretroviral Therapies (HAARTs), the morbidity and mortality due to AIDS remains alarmingly high. Most of the failure to contain the HIV virus has been attributed to several factors including drug resistance [1-3]. Attempts to control the spread of the virus through the development of a vaccine are also yet to yield any viable vaccine candidates [4,5]. The search for new and more active agents against the HIV virus as such remains a priority as existing therapies can become ineffective due to resistance or toxicity [6]. Natural products, especially from plants, have been shown to possess anti-HIV activity [7]. The michellamine series of alkaloids, derivatives of betulinic acid and other classes of natural products are amongst some of the molecules isolated from plants with anti-HIV activity [8-10]. Jamaica is endowed with several medicinal plant species and is credited for being the source of the *Catharanthus roseus* plant which yielded vinca alkaloids that were later developed for the treatment of leukemia[11,12]. Our research has recently identified a number of Jamaican plants with varying degrees of both anticancer and anti-HIV activity [13,14].

*Petiveria alliacea* L. is commonly known in Jamaica as the “Guinea Hen Weed”, and is claimed to have several medicinal properties. It is used in folk medicine to enhance memory and in the treatment of the common cold, flu, other viral or bacterial infections, inflammation, diabetes, and cancer [15-18]. Studies have confirmed the anticancer potential of guinea Hen Weed in-vitro as well as in-vivo [19,20]. Dibenzyl trisulfide (DTS) (Fig. 1) was identified as the major metabolite of *P. alliacea* [21], and closer investigation of its biological properties revealed its anticancer activity, playing a regulatory role in the signal transduction of the mitogen-activated protein kinase (MAPK) pathway [22]. Recent studies have confirmed the involvement of DTS in the MAPK pathway potently inhibiting the ribosomal s6 kinase (RSK), revealing that this might be its mechanisms of action and conferring it with broad cancer therapeutic potential [23]. The first study on the antiviral activity of Guinea Hen Weed was in regard to its activity against the bovine viral diarrhea virus [24]. This paper is focused on the anti-HIV-1 activity of Guinea Hen Weed and its anticancer metabolite DTS.

**Fig. 1. Chemical structure of dibenzyl trisulfide (DTS)**

**2. MATERIALS AND METHODS**

**2.1 Chemicals and Reagents**

Major chemicals and reagents where obtained as follows: Dibenzyl trisulfide, 98% purity (International Laboratory, USA); Poly rA:oligodT template: primer (GE Healthcare); DE81 filter-mats (Wallac); sodium phosphate buffer or 2X SSC (Life Technologies); MTS CellTiter®96 Reagent (Promega).

**2.2 Plant Collection and Extraction**

Plant collection: The leaves of *P. alliacea* were collected in Jamaica. A specimen of the plant was identified at the University of the West Indies, Mona Herbarium (deposited under Accession Numbers: IJ 35931). The collected plant material was air dried away from direct sunlight and pulverized.
Plant extraction: *Petiveria alliacea* powder extracted with ethyl acetate and methanol at room temperature for 48 hrs. The filtered solutions were dried in a rotary evaporator. Table 1 presents the details of the extraction and yields.

### 2.3 Extract Handling

A sample of each extract was dissolved in DMSO and stored at -20ºC. The samples were subsequently diluted as necessary prior to use in the anti HIV assay.

### 2.4 Bioassay

#### 2.4.1 Drugs, cells and virus

Stocks of crude drug made from *P. alliacea* were prepared in DMSO in a stock solution of 10mg/ml. Dilutions from the stock were made in RPMI medium and the final concentration of DMSO in the cultures was always <0.1% to avoid cell toxicity. Peripheral blood mononuclear cells (PBMCs) were separated from buffy coats of healthy donors. Virus used was HIV-1 strains JR-CSF.

#### 2.4.2 Infectivity assays

Three-day phytohemagglutinin (PHA) stimulated PBMCs from at least two normal donors were mixed together. Equivalent number of cells were infected with HIV-1 JRFL using a multiplicity of infection (m.o.i) of 0.001 for 3 h. Infected cells were washed to remove non-adsorbed virus and plated in fresh medium containing IL-2 and various concentrations of drugs. Cells were plated in 96-well plates (2X10^5 cells/well in 200 µl). Two days after infection, half of the medium was replenished with fresh medium plus drugs. Seven days after infection, supernatants were assayed for virus production by measuring HIV Reverse Transcriptase (RT) activity, and cell viability evaluated.

#### 2.4.3 Reverse transcriptase activity assay

For measuring RT activity, we used a previously described microtiter plate-based assay [25]. Tritiated thymidine triphosphate (³H-TTP, 80 Ci/mmol, NEN) was received in 1:1 dH₂O: Ethanol at 1 mCi/mL. Poly rA:oligodT template: primer was prepared as a stock solution by combining 150 µL poly rA (20 mg/mL) with 0.5 mL oligodT (20 units/mL) and 5.35 mL sterile dH₂O, followed by aliquoting (1.0 mL) and storage at -20ºC. The RT reaction buffer was prepared fresh on a daily basis and consisted of 125 µL 1.0 M EGTA, 125 µL dH₂O, 125 µL 20% Triton X100, 50 µL 1.0 M Tris (pH 7.4), 50 µL 1.0 M DTT, and 40 µL 1.0 M MgCl₂. The final reaction mixture was prepared by combining 1 part ³H-TTP, 4 parts dH₂O, 2.5 parts poly rA:oligodT stock and 2.5 parts reaction buffer. Ten microliters of this reaction mixture were placed in a round bottom microtiter plate and 15 µL of virus containing supernatant is added and mixed. The plate was incubated at 37ºC for 60 minutes. Following incubation, the reaction volume was spotted onto DE81 filter-mats, washed 5 times for 5 minutes each in a 5% sodium phosphate buffer or 2X SSC, 2 times for 1 minute each in distilled water, 2 times for 1 minute each in 70% ethanol, and then dried. Incorporated radioactivity (counts per minute, CPM) was quantified using standard liquid scintillation techniques. For measuring p24 levels, we used a commercial p24 ELISA assay (Coulter), as previously described [14].

#### 2.4.4 PBMC cytotoxicity assays

At assay termination, plates were stained with the soluble tetrazolium-based dye, MTS Cell Titer®96 Reagent, to determine cell viability. MTS is metabolized by the mitochondria enzymes of metabolically active cells to yield a soluble formazan product, allowing the rapid quantitative analysis of cell viability and compound cytotoxicity. Briefly, 20-25 µL of MTS reagent was added per well, and the microtiter plates were then incubated 4-6 hrs at 37ºC, 5% CO₂. Adhesive plate sealers were used in place of the lids, the sealed plate was inverted several times to mix the soluble formazan product and the plate was read spectrophotometrically at 490/650 nm with a Molecular Devices SpectraMax Plus plate reader.

### 3. RESULTS AND DISCUSSION

The yields of the different extracts of *P. alliacea* are presented in Table 1. The methanol extract had the highest yield (4.646%) and the ethyl acetate extract had a yield of 0.370% which was only about 8% of the methanol extract yield.

The results of the *P. alliacea* reverse transcriptase (RT) activity against the HIV-1 JR-CSF strain are presented in Table 2 and Fig. 2. The cytotoxic effects of these extracts on PBMCs were measured simultaneously in the assay to ascertain that RT effect was not influenced by.
cytotoxicity. The methanolic extract demonstrated higher activity compared to the ethyl acetate extract in the RT assay, with an EC_{50} of 21.60 μg/ml and an IC_{50}>100 μg/ml. The SI for the methanol extract was >4.62, compared to an SI of only >1.47 for the ethyl acetate extract. Similar results were obtained by measuring p24 levels in the culture supernatants (data not shown).

In contrast, DTS was active against HIV-1 JR-CSF but had higher cytotoxicity compared to the crude extracts. Being a pure molecule, DTS is as such unlikely to be regarded as a possible anti-HIV-1 agent given its poor selectivity.

The implication is that there is possibly a different molecule(s) responsible for the anti-HIV-1 activity of the crude extracts of *P. alliacea*. The fact that the methanolic extract exhibits antiviral activity in the RT assay suggests that the anti-HIV-1 molecule(s) might be of mid to high polarity given that DTS is highly nonpolar.

### Table 1. Extraction of *P. alliacea* using different solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Volume (mL)</th>
<th>Amount (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>500</td>
<td>25</td>
<td>1.162</td>
</tr>
<tr>
<td>Ethyl</td>
<td>500</td>
<td>25</td>
<td>0.093</td>
</tr>
</tbody>
</table>

### Table 2. Anti HIV-1 activity of extracts of *P. alliacea* L. and dibenzyl trisulfide (DTS)

<table>
<thead>
<tr>
<th>Extract/compound</th>
<th>Activity against</th>
<th>Selectivity index (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC_{50} (μg/ml)</td>
<td>IC_{50} (μg/ml)</td>
</tr>
<tr>
<td>GHW</td>
<td>21.60</td>
<td>&gt;100</td>
</tr>
<tr>
<td>GHW-E</td>
<td>68.00</td>
<td>&gt;100</td>
</tr>
<tr>
<td>DTS</td>
<td>5.60</td>
<td>9.53</td>
</tr>
<tr>
<td>AZT</td>
<td>0.005</td>
<td>&gt;1</td>
</tr>
</tbody>
</table>

SI = IC_{50}/EC_{50}. EC_{50} and IC_{50} values were determined by variable slope non-linear regression analysis of the plotted data using GraphPad Prism software. *P. alliacea* extracts: ethyl acetate (GHW-E) and methanol (GHW).
While the possible mechanism of action of \textit{P. alliacea} extracts against the HIV-1 virus remains to be discovered, data from previous studies indicate that \textit{P. alliacea} extracts do decrease the expression of cyclophylin A in a breast carcinoma cell line (4T1) [19]. This is very interesting because human cyclophins A and B are host cell proteins that bind specifically to the HIV-1 Gag polyprotein p55\textsuperscript{gag} \textit{in vitro} and drug-induced reductions in virion-associated cyclophilin A levels have been shown to be accompanied by reductions in HIV-1 virion infectivity, indicating that the association is functionally relevant for HIV-1 infectivity [26,27]. Based on the findings that cyclophins are important for viral capsid assembly, cyclophins are as such considered druggable targets in antiviral therapy [28,29]. Other studies that lend support to the therapeutic potential of \textit{P. alliacea} against HIV-1/AIDS is the fact that this plant is credited for having immunomodulatory properties [21,30]. The human immune system is used to fight off infections and as such it is one of the first targets attacked by the HIV-1 virus guaranteeing its survival in the human system [31,32]. To combat the HIV infection and slow the progression to full blown AIDS, immunotherapy is often recommended as a therapeutic strategy [32-34]. Stimulation of natural immune response to infections are considered necessary for the development of an HIV vaccine [35]. An antioxidant metabolite was also isolated and characterized from \textit{P. alliacea} yielding further evidence of the immunomodulatory properties of this plant [36].

4. CONCLUSION

In conclusion, the plethora of evidence on the medicinal properties of \textit{P. alliacea} keeps growing, making this plant one of the most popular medicinal plants in Jamaica, and the Caribbean Islands in general. The anti HIV-1 activity of \textit{P. alliacea} is reported herein for the first time. Given that this plant is already being marketed in Jamaica in capsules and as a tea, it is possible that HIV infected persons taking the supplements for other reasons might be benefiting from the anti HIV effects of this plant without knowing. The existing information on its possible mechanism of action, combined with its reported effects on the immune system, makes this plant a good candidate for the potential development of a new therapy to be used alone or in combination with other agents against HIV-1. The activity of \textit{P. alliacea} against reference HIV-1 strain JRCSF, which uses CCR5 as a co-receptor, is important in that CCR5 strains are the predominantly transmitted and generally persist during the course of infection [37-40]. In summary, our data suggest that \textit{P. alliacea} may have therapeutic potential against HIV-1 and further studies are required to demonstrate the extent of its activity.

CONSENT

Not applicable.

ETHICAL CLEARANCE

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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