



Anti HIV-1 Activity of the Crude Extracts of *Guaiaacum officinale* L. (Zygophyllaceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Conceived experiment and designed the experiments by authors HICL, NJT and JB. Performed experiments by authors NJT and AH. Analyzed the data by authors AH, NJT and CTW. Wrote the paper by authors HICL, NJT and JB. All authors read and approved the final manuscript.

Short Research Article

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ABSTRACT

Aim: Jamaica is rich in medicinal plants. *Guaiaacum officinale* is the "National Flower", with reported uses in folk medicine for the treatment of various conditions including inflammation. In our search for plants with anticancer and anti-infective properties, we evaluated *Guaiaacum officinale* for activity against HIV-1.

Methodology: The leaf, seed and twig extracts of *G. officinale* were screened for anti HIV-1 properties in primary peripheral blood mononuclear cells (PBMCs) infected with the reference HIV-1 BaL strain.

Results: All the tested extracts inhibited HIV-1 p24 production by infected cells, with EC₅₀ concentrations of 22.35µg/ml, 23.42µg/ml and 25.04µg/ml, respectively for the leaf, seed and twig extracts. As comparison, Betulinic acid had an EC₅₀ value of 27.50µg/ml. The tested extracts had IC₅₀/EC₅₀ selectivity index (SI) values of ≥ 3, which compared favorably to Betulinic acid SI value of 1.09.

Conclusion: The results of this study suggest that extracts of *G. officinale* may provide leads for the discovery of new drug agents against HIV-1.

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1. INTRODUCTION

A number of natural products, mainly from plants have been shown to possess anti HIV activity [1]. They have attracted the attention of the US National Cancer Institute (NCI), resulting in the screening of thousands of plant extracts in search of effective drugs against HIV [1,2]. The michellamine series of alkaloids and derivatives of betulinic acid are amongst some of the molecules isolated from plants with anti HIV activity [3,4]. To contribute to the search for new drugs, our group has focused on the screening of Jamaican plants for their anticancer as well as anti HIV properties. This paper presents our findings on HIV-1 inhibition by extracts of *Guaiacum officinale* L., the Jamaican National Flower.

Guaiacum officinale L. belongs to the Zygophyllaceae family and is commonly known as *Lignum vitae*. The plant is known to have a number of medicinal uses in folk medicine. For example, mixtures containing barks from *G. officinale* and Maubi bark have been used in traditional Virgin Islands bush medicine to relieve fish poisoning [5]. The flowers and leaves are used to make a tea with energy restorative properties and as abortifacient [6,7]. Elsewhere, there is abundant anecdotal evidence that the resins and extracts of *G. officinale* have antiinflammatory properties with activity against arthritis, gout and sciatica condition [8]. Furthermore, the resins of the plant are used in traditional medicines in Pakistan to cure angina, tonsillitis, rheumatoid arthritis, mucous membrane diseases and abnormalities of metabolic processes [9].

In terms of its phytochemistry, *G. officinale* is known to be a rich source of saponins [10]. Saponins are credited for several biological activities including membrane-permeabilising, immunostimulant and hypocholesterolaemic properties [11]. Several mono and bidesmosidic saponins having akebonic acid and oleanolic acid as genins have previously been isolated and reported from this plant [12,13]. A new triterpenoidal saponin, guaianin N was isolated from the butanolic extract of the flowers of *G. officinale* [10]. Guaianin N showed antibacterial activity against *Pseudomonas pseudomaliae* as well as brine shrimp toxicity. Guaiacin A and B, two new saponins have also been isolated from the leaves of *G. officinale* [14].

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

Plant collection: The leaves of *G. officinale* were collected at Eden Garden, Jamaica. A voucher specimen of the plant was identified at the University of the West Indies, Mona Herbarium (deposited under Accession Numbers: 35724 and 35725). The collected plant material was air dried away from direct sunlight and pulverized.

Plant extraction: The leaves, seeds and twigs of ground *G. officinale* were extracted with methanol at room temperature for 48 hrs. The filtered solutions were dried in a rotary evaporator. Table 1 presents the details of the extraction process.

To obtain the ethyl acetate fraction, 1.0 g of the crude methanolic extract was extracted with ethyl acetate 3x 20 mL. The extract was concentrated in a speedvac yielding 0.214 g of the ethyl acetate soluble portion.

Table 1. Extraction of different parts of *G. officinale*

| Plant part | Solvent | Volume | Amount (kg) | Yield (g) | % Yield |
|------------|----------|--------|-------------|-----------|---------|
| | Methanol | 6 L | 1.20 | 58.40 | 4.87 |
| Seeds | Methanol | 2 L | 0.35 | 25.31 | 7.23 |
| Twigs | methanol | 3 L | 0.50 | 18.20 | 3.64 |

2.2 Extract Handling

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

2.3 Bioassay

2.3.1 Drugs, cells and virus

Stocks of crude drug made from *G. officinale* 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 HIV-1 seronegative donors (New York Blood Center, NY) by density centrifugation over Ficoll-Hypaque (Sigma). Briefly, the buffy coat suspension was diluted 1:2 with PBS. Then, 35ml were layered onto 15ml of Ficoll Paque (Sigma) and centrifuged for 25 min at 400X g. The white interphase containing the PBMCs was collected and washed three times with PBS. PBMCs were cultured in 5% CO₂ at 37°C, in RPMI medium supplemented with L-glutamine (300 mg/ml), penicillin/streptomycin (10 U/ml) and 10% heat inactivated fetal bovine serum (FBS); herein referred to as RPMI-10 medium.

For infection of PBMCs, we used the CCR5 tropic HIV-1 reference strain HIV-1 BaL [15] Prior to infection, PBMCs were first activated by culture in RPMI-10 medium containing 2.5 µg/ml of the mitogen phytohemagglutinin (PHA; Roche, Indianapolis, IN) for 3 days. PHA activation renders PBMCs highly susceptible to productive infection with HIV-1. Following 3 days, PBMCs were infected by incubation with virus at a multiplicity of infection (MOI) of 0.001 for 2 hours. PBMCs were then washed three times with PBS to remove residual virus and seeded in 96-well flat-bottom plates at a density of 2 x 10⁵ PBLs/200 µl in RPMI-10 supplemented with 10 units/ml of the growth factor Interleukin 2 (IL-2; Roche) and drugs. IL-2 addition is required for optimal proliferation of cultured PBMCs. Following 3 days of culture, half of the medium was discarded and replenished with fresh medium containing IL-2 and drugs at the same final concentration as before. This step helps maintain viability of cultured cells and drug activity. On day 7, HIV-1 p24 protein production in the culture supernatant was measured using a commercial ELISA kit (Coulter, Hialeah, FL) and cell viability using a colorimetric MTT kit (Roche). The MTT test is based on the reduction of the yellow colored MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to blue formazan by mitochondrial dehydrogenases. The quantity of formazan produced (absorbance at 490 nm) is directly proportional to the number of living cells. Briefly, cell aliquots were seeded in 96-well plates (100 µl) and incubated with 10 µl of MTT solution for 4 h at 37°C. A solubilization solution (50 µl) was added and plates incubated overnight at 37°C. MTT conversion to formazan by mitochondrial dehydrogenase was assayed by optical density at 490 nm measured in an ELISA plate reader. MTT assays were also

conducted on uninfected PHA-activated PBMCs cultured in the presence of serial dilutions of each drug to assess toxicity.

3. RESULTS AND DISCUSSION

The results of the yields of the different extracts of *G. officinale* are presented in Table 1. The seed extract had the highest yield (7.23%), followed by leaf (4.87%), and twigs (3.64%). Extraction of the methanolic leaf extract with ethyl acetate yielded 0.214 g (21.40%) of an ethyl acetate soluble fraction.

The results of the anti HIV-1 activity of methanolic leaf, seed and twig extracts of *G. officinale* are presented in Table 2. The results include also the cytotoxicity effects of these extracts on PBMCs. In a preliminary attempt to determine if the anti HIV-1 activity could be enhanced by fractionation of the extracts, the ethyl acetate soluble portion of the methanol leaf extract was screened and the results are indicated on Fig. 1. The ethyl acetate soluble portion demonstrated more potent activity compared to the parent methanolic extract, with an EC_{50} of 6.79 μ g/ml and an IC_{50} of 29.24 μ g/ml. The SI for the ethyl acetate fraction was 4.30, compared to an SI of only 1.09 for the reference molecule betulinic acid (BA).

Table 2. Anti HIV-1 activity of crude extracts of *G. officinale* L.

| Extract/compound | Activity against BaL | | |
|------------------|-------------------------|-------------------------|------------------------|
| | EC_{50} (μ g/ml) | IC_{50} (μ g/ml) | Selectivity index (SI) |
| | 22.35 | 75.96 | 3.39 |
| Seed | 23.42 | 71.63 | 3.05 |
| Twig | 25.04 | 78.60 | 3.13 |
| Betulinic acid | 27.52 | 30.06 | 1.09 |

$SI = IC_{50}/EC_{50}$. EC_{50} and IC_{50} values were determined by variable slope non-linear regression analysis of the plotted data using GraphPad Prism software.

The three-fold greater activity of the ethyl acetate soluble portion of the methanolic leaf extracts suggests that the active compound (s) in the leaf extract is located in the ethyl acetate portion of the leaf extract. As such, it is expected that the active compound(s) will have mid to low polar solubility characteristics. Betulinic acid was used as a reference compound given previous reports on its anti-HIV activity [4,16], as well as structural similarity to triterpenes that might be found in *G. officinale* [10]. However, all the extracts of *G. officinale* showed higher anti HIV-1 activity than Betulinic acid. The anti-HIV activity of the ethyl acetate fraction of *G. officinale* is very close to results obtained with extracts from other medicinal plants based on the p24 elixir assay including *Sophora flavescens* [17], *Argemone Mexicana*, *Asparagus Mexican*, *Butea monosperma*, *Cassia occidentalis*, *Coleus forskohlii*, *Glycyrrhiza glabra*, *Terminalia arjuna*, *Tinospora cordifolia* [18], *Rhus chinensis* [19], *Bulbine alooides*, *Crinum macowanii*, *Hypoxis sobolifera* and *Leonotis leonurus* [20].

In summary, we have demonstrated that the leaf, seed and twig extracts of *G. officinale* have activity against HIV-1 BaL, a reference HIV-1 strain that uses CCR5 as coreceptor for infection. Strains of HIV-1 that use CCR5 are the most persistent and predominantly transmitted ones [21-24]. Thus, our data suggest that *G. officinale* extracts could help control HIV-1 replication in infected patients. Moreover, their use in microbicide formulations could help prevent sexual transmission of HIV-1 [25,26].

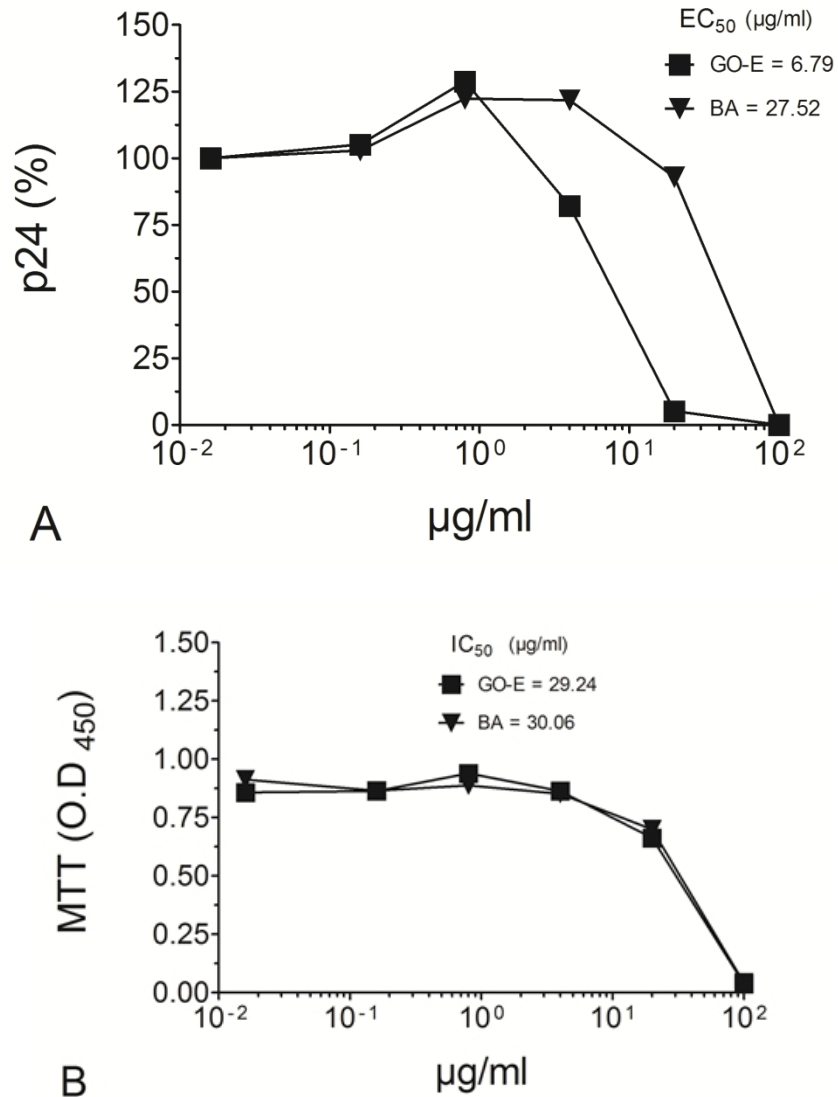


Fig. 1. GO-E: *G. officinale* ethyl acetate extract; BA: Betulinic acid. A) *In-vitro* anti HIV-1 activity of the ethyl acetate portion of the methanol leaf extract of *G. officinale* was determined by measuring the p24 levels on cultures of PBMCs infected with HIV-1 BaL for 7 days. EC₅₀ values were generated using a dose response curves utilizing the Graphpad Prism software and B) Cytotoxicity activity was concurrently determined for the extract against PBMC cells and IC₅₀ values determined using the Graphpad Prism software. Betulinic acid was used as a reference compound. Data are representative from 1 of 2 independent experiments.

4. CONCLUSION

Together, our data suggest that extracts of *G. officinale* may provide leads for the discovery of new anti HIV-1 drugs and thus warranting further investigation.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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