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# Anti-HIV-1 Activity of of *Dimocarpus longan* Lour. Extracts and the Main Chemical Content

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**Abstract**

The present study is carried out for the evaluation of anti-HIV-1 effect of *Dimocarpus longan* extracts and also the investigation of the phytoconstituents from the extracts. Chloroform, ethyl acetate and methanol 80% extracts of *Dimocarpus longan* aerial parts were tested for their anti-HIV-1 activity using the syncytia formation assay. Ethyl acetate from *Dimocarpus longan* aerial parts extract has shown anti-HIV-1 activity and the other extracts were less active as anti-HIV-1 agents. Phytochemical analysis of the plant extracts proves the presence of triterpenes, flavonoids, tannins and carbohydrates. In conclusion the results prove that *Dimocarpus longan* extracts have drug ability as anti- HIV-1 agents.

**Keywords**: *Dimocarpus longan*, aerial parts, cytotoxicity, anti-HIV-1 activity, phytoconstituents.

## Introduction

The human Immunodeficiency virus type 1 (HIV-1) is an aetiological agent for Acquired Immunodeficiency Syndrome (AIDS). Medicinal plants as potential sources of new active agents not only combine the advantage of being relatively non-toxic and hence more tolerable than rationally designed drugs, but also represent an affordable and valuable source of pharmacologically active substances that can be made sufficiently available through cultivation [1]. Plant substances are especially explored due to their amazing structural diversity and their broad range of biological activities.

Several plant extracts have been shown to possess activity against HIV by inhibiting various viral enzymes [1]. In our screening for new anti-HIV-1 agents from plants, *Dimocarpus longan* Lour. (soapberry family) is commonly known as Longan and it is native to Southeast Asia, such as China, Taiwan and Thailand. It is a tropical tree that produces edible fruit. The fruit of *Dimocarpus longan* was used as a traditional Chinese medicine for different treatments, such as promoting blood metabolism, soothing nerves, and relieving insomnia (2). Previous phytochemical and pharmacological studies of *Dimocarpus longan* showed that longan pericarp contain high amounts of bioactive compounds, such as phenolic acids, flavonoids, and polysaccharides and exhibit antibacterial, antiviral, antioxidant, anti-inflammatory, and anticarcinogenic properties (3, 4). We aimed to evaluate anti-HIV-1 effect of *Dimocarpus longan* aerial parts extracts and also investigate the chemical content of the bio-extracts.

## Experimental

### Materials and methods

### Plant Material

The aerial parts of *Dimocarpus longan* were collected from Al-Zohiriya garden, Giza, Egypt in May 2011. The plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereez Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen is deposited in the herbarium of Al-Zohiriya garden, Giza, Egypt.

### Reagents

AZT (3'-azido-3'-deoxythymidine) was purchased from Sigma. All extracts were dissolved in DMSO. AZT was dissolved in RPMI-1640 and stored at -20$^{o}$C. HEPES (N-2(2-Hydroxyothyl) piperazine-N'-(2-ethanesufonic acid), MTT (3, (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide), DMF (N, N'- Dimethyl formamine), Penicillin, Streptomycin sulfate, Glutamine were purchased from Sigma; 2-ME (2-Mercaptoethanol) was purchased from Bio-Rad. RPMI-1640 and fetal bovine serum (FBS) were purchased from Gibco.

### Cells and virus

C8166 cells and HIV-1IIIB were kindly donated by Medical Research Council, AIDS Regent Project. The cells were maintained at 37$^{o}$C in 5% CO2 in RPMI-1640 medium supplemented with 10% heat-inactivating FBS (Gibco). HIV-1IIIB was prepared from the supernatants of H9/HIV-1IIIB cells. The 50% HIV-1 tissue culture infectious dose (TCID50) in C8166 cells was determined and calculated by Reed and Muench (5). Virus stocks were stored in small aliquots at -70$^{o}$C.

### Cytotoxicity assay

The cellular toxicity of the extracts on C8166 cells was assessed by MTT colorimetric assay. Briefly, 100µl of $4\times10^{5}$ cells were plated into 96-well plates, 100 µl of various

concentrations of compounds was added and incubated at 37°C in a humidifed atmosphere of 5% $CO_2$ for 72 h. 100 µl of supernatant was discarded, MTT reagent was added and incubated for 4 h and 100µl 50% DMF-20% SDS was added. After the formazan was dissolved completely, the plates were read on a Bio-Tek ELx 800 ELISA reader at 570 nm/630 nm. 50% cytotoxicity concentration ($CC_{50}$) was calculated (6).

**Inhibition of syncytia formation**

The effect of the extracts on acute HIV-1 infectivity was measured by the syncytia formation assay (7). In the presence or absence of various concentrations of samples, $4\times10^4$ C8166 cells were infected with HIV-1 at a multiplicity of infection (MOI) of 0.015, and cultured in 96-well plates at 37°C in 5% $CO_2$ for 3 days. AZT was used as a positive control. At 3 days post-infection, the cytopathic effect (CPE) was measured by counting the number of syncytia (multinucleated giant cell) in each well of 96-well plates under an inverted microscope (100×). The inhibitory percentage of syncytia formation was calculated by the percentage of syncytia number in sample-treated culture compared to that in infected control culture 50% effective concentration ($EC_{50}$) was calculated according to the method described by Reed and Muench (5), 50% cytotoxic concentration ($CC_{50}$) and 50% effective concentration ($EC_{50}$) was determined from dose–response curve. Therapeutic index (TI of anti-HIV activity is $CC_{50}/EC_{50}$

1. Cell viability (% of control) = $(OD_{test}-OD_{blk})/(OD_{ctrl} - OD_{blk})\times100$
2. CPE inhibition(%) = $(1-CPE_{test}/CPE_{ctrl})\times100$

**Preparation of the extracts**

Finely ground aerial parts from *Dimocarpus longan* (550) g were extracted with chloroform, ethyl acetate and methanol 80% solvents by maceration. Each extract was concentrated to dryness to yield 12.5 g of chloroform, 9 g of ethyl acetate and 32 g of methanol 80% extract. Each extract was tested for the presence of the phytoconstituents according to the following standard tests, Molisch's test for carbohydrates, Shinoda test for flavonoids, forth test for saponins, Salkowski 's for terpenes and sterols, $FeCl_3$ and Mayer's reagents for detecting of tannins and alkaloids, respectively (8-10).

## Results and Discussion

The results showed that *Dimocarpus longan* aerial parts extracts were minimal toxic where chloroform extract of *Dimocarpus longan* was less toxic than the other two extracts. All the extracts have drug ability as anti-HIV-1 agents where ethyl acetate extract was more active than chloroform and methanol 80% extracts as an anti-HIV-1 agents, (Table 1, Table 2 and Table 3). Phytochemical analysis of *Dimocarpus longan* aerial parts extracts is shown in table 4. The phytochemical analysis has shown the presence of triterpenes, flavonoids, tannins and carbohydrates. Cytotoxicity of the *Dimocarpus longan* aerial parts extracts was carried out by using MTT colormetric methods, *Dimocarpus longan* aerial parts extracts were minimal toxic and showed

anti-HIV-1 activity. Chloroform extract of *Dimocarpus longan* had less cytotoxic effect, it was significantly different from that of the other extracts (Table 1). The anti-HIV-1 activity assay was performed by synctia formation. Ethyl acetate extract of *Dimocarpus longan* aerial parts showed anti-HIV-1 activity and its therapeutic index (TI) value was the higher than that of methanol (80%) and chloroform extracts (table 2, table 3) with comparison with AZT. These results may be explained by the presence of phytochemicals in ethyl acetate extract as triterpenes and/or sterols, tannins, flavonoids and carbohydrates (Table 4). Triterpenes as oleanolic acid was identified as an anti-HIV principle which was isolated from several plants, including *Rosa woodsii* (leaves), *Prosopis glandulosa* (leaves and twigs), *Phoradendron juniperinum* (whole plant), *Syzygium claviflorum* (leaves), *Hyptis capitata* (whole plant), and *Ternstromia gymnanthera* (aerial part). It inhibited HIV-1 replication in acutely infected H9 cells with an EC50 value of 1.7 microg/mL, and inhibited H9 cell growth with an $IC_{50}$ value of 21.8 microg/mL with therapeutic index (T.I) = 12.8, also ursolic acid showed anti-HIV activity (EC50 2.0 microg/mL), but it was slightly toxic ($IC_{50}$ 6.5 microg/mL, (TI) = 3.3 (11). Tannins inhibit HIV-1 entry by targeting gp41 (12), since tannin is a non-uniform polyphenolic compound. Tannins also inhibit fusion of HIV-$1_{IIIB}$-infected of H9 cells with uninfected MT-2 cells and so inhibits replication of HIV-1 by targeting the viral proteins that mediate the late steps of HIV replication (13), as well luteolin cripples HIV-1 by abrogation of Tat function (14), as well some phenolic compounds (flavonoids and tannins) have anti-HIV-1 activity (15).

**Table 1:** Cytotoxicity of the extracts of *Dimocarpus longan* in C8166 cell

| **Extracts** | **Concentration (µg/ml)** | **Cell viability ±SD** | **$CC_{50}$ (µg/ml)** |
|---|---|---|---|
| Chloroform | 200 | 2.29 ± 0.45 | 24.32 |
| | 40 | 32.49 ± 0.18 | |
| | 8 | 89.11 ± 0.71 | |
| | 1.6 | 91 ± 9.27 | |
| | 0.32 | 95.1 ± 3.3 | |
| Ethyl acetate | 200 | 40.88 ± 2.76 | 161.02 |
| | 40 | 108.6 ± 4.73 | |
| | 8 | 113.39 ± 4.01 | |
| | 1.6 | 109.79 ± 1.07 | |
| | 0.32 | 113.01 ± 0.09 | |
| Methanol 80% | 200 | 37.98 ± 1.16 | 154.87 |
| | 40 | 113.64 ± 0.62 | |
| | 8 | 107.08 ± 1.16 | |
| | 1.6 | 109.98 ± 0.8 | |
| | 0.32 | 104.81 ± 6.51 | |

| | | | |
|---|---|---|---|
| AZT | 2000 | 33.59 ± 0.44 | 1043 |
| | 400 | 74.19 ± 0.53 | |
| | 80 | 91.14 ± 4.33 | |
| | 16 | 82.07 ± 11.32 | |
| | 3.2 | 77.81 ± 0.71 | |
| | 0.64 | 86.76 ± 8.05 | |

**Table 2:** Anti-HIV activity of the extracts of *Dimocarpus longan* in C8166 cell

| **Extracts** | **Concentration (µg/ml)** | **Inhibition ± SD (%)** | **$EC_{50}$ (µg/ml)** |
|---|---|---|---|
| Chloroform | 200 | 100.00 ± 0.00 | 11.82 |
| | 40 | 100.00 ± 0.00 | |
| | 8 | 34.00 ± 2.83 | |
| Ethyl acetate | 200 | 100.00 ± 0.00 | 42.55 |
| | 40 | 48.00 ± 1.89 | |
| Methanol 80% | 200 | 100.00 ± 0.00 | 65.41 |
| | 40 | 28.00 ± 3.77 | |
| AZT | 2000 | 100.00 ± 0.00 | 2.27 |
| | 400 | 100.00 ± 0.00 | |
| | 80 | 100.00 ± 0.00 | |
| | 16 | 88.78 ± 1.44 | |
| | 3.2 | 39.29 ± 0.72 | |

**Table 3:** The summary of cytotoxicity and anti-HIV-1 activities of *Dimocarpus longan* extracts

| **Extracts** | **Method** | **$CC_{50}$ (µg/ml)** | **$EC_{50}$ (µg/ml)** | **Therapeutic index (TI)** |
|---|---|---|---|---|
| Chloroform | MTT | 24.32 | — | 2.06 |
| | CPE | — | 11.82 | |
| Ethyl acetate | MTT | 161.02 | — | 3.78 |
| | CPE | — | 42.55 | |
| Methanol 80% | MTT | 154.87 | — | 2.37 |
| | CPE | — | 65.41 | |
| AZT | MTT | 1043µg/ml | — | 459471 |
| | CPE | — | 2.27 ng/ml | |

**Table 4:** Phytochemical Analysis from *Dimocarpus longan* aerial parts extracts

| **Constituents** | **Chloroform** | **Ethyl acetate** | **Methanol 80%** |
|---|---|---|---|
| Triterpenes and /or Sterols | + | + | + |
| Carbohydrates and/or glycosides | - | + | + |
| Flavonoids | - | + | + |
| Coumarins | - | - | - |
| Alkaloids and/or nitrogenous compounds | - | - | - |
| Tannins | - | + | + |
| Saponins | - | - | - |

(+) presence of constituents, (-) absence of constituents

## Conclusion

We extracted *Dimocarpus longan* aerial parts with chloroform, ethyl acetate and methanol 80% solvents by maceration method and each extract was tested for its ability to act as anti-HIV-1 agent. All the extracts have drug ability to act as anti-HIV-1 agents where ethyl acetate extract was the most active extract as an anti-HIV-1 agent and this may be explained by the presence of phytoconstituents as flavonoids, triterpenes, tannins and carbohydrates in ethyl acetate extract and thus *Dimocarpus longan* ethyl acetate extract can act as anti-HIV-1 agent.

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## Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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