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Research Article

Phytochemical Screening of the Polar Extracts of *Carica papaya* Linn. and the Evaluation of their anti-HIV-1 Activity

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***Abstract*— This research work deals with the evaluation of anti-HIV-1 effect of *Carica papaya* aerial parts polar extracts and also the investigation of the chemical content from the polar extracts of the plant. The methanol and aqueous extracts of *Carica papaya* were tested for their anti-HIV-1 activity using the syncytia formation assay. Methanol and aqueous extracts of *Carica papaya* aerial parts showed activity as anti-HIV-1 agents, both of the extracts Therapeutic index (TI) of 5.51 and 7.13 compared with the standard drug. Phytochemical analysis of both the extracts proves the presence of phytocomponents as flavonoids, tannins, alkaloids, carbohydrates and triterpenes. The results have shown that *Carica papaya* methanol and aqueous extracts have drug ability as anti- HIV-1 agents.**

***Index Terms*— *Carica papaya*, aerial parts, cytotoxicity, anti-HIV-1 activity, phytoconstituents.**

I. INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a clinical syndrome that is the result of infection with human immunodeficiency virus (HIV), which causes profound immuno-suppression. HIV-1 is the cause of the world epidemic and is mostly commonly referred as HIV. It is a highly variable virus, which mutates readily. The herbal medicines are frequently used as an alternate therapy for inhibitory effects on HIV replication. Medicinal plants and their products may be explored as a source of new anti-HIV-1 agents. In addition, herbal medicines have some advantages such as fewer side effects, better tolerance, relatively less expensive and freely available **[1]**. *Carica papaya* Linn. (family: Caricaceae) known as pawpaw tree is a vegetable fruit widely distributed throughout the world, mostly grow in tropics and it grows up to 5 to 10 m tall. Its leaves are large, 50-70 cm in diameter, deeply palmately lobed with 7 lobes **[2]**. The ripe fruit is edible and is usually eaten raw, without the skin or seeds. The unripe green fruit (which is a rich source of vitamin A) can be eaten cooked, usually in curries, salads and stews as used in Thai cuisine **[3]**. The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present **[4]**. It has a good effect as a remedy in dyspepsia and kindred ailments. The juice has been in use on meat to make it tender **[4]**. The seed is used for intestinal worms when chewed. The root is chewed and the juice swallowed for cough, bronchitis, and other respiratory diseases. The unripe fruit is used as a remedy for ulcer and impotence **[5]**. Previous studies on biological activities of *Carica papaya* parts, extracts and isolated compounds showed that the latex and root extracts inhibited *Candida albicans* while extracts of pulp and seeds showed bacteriostatic properties against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, and *Entamoeba histolytica* **[6]**. Its root aqueous extract has equally been shown to have purgative effect **[7]**. In another study, the hypoglycemic and hypolipidemic effects of the aqueous seed extract of *Carica papaya* was reported and the LD_{50} of the oral toxicity was estimated to be greater than 2000 mg/kg/oral route *in vitro* **[8]**. The present study was carried out to evaluate the anti-HIV-1 effect of polar extracts from *Carica papaya* aerial parts and also to investigate the main phytoconstituents in the polar plant extracts.

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II. MATERIALS AND METHODS

Plant Material

The aerial parts of *Carica papaya* were collected from the Agricultural Research Center, Giza, Egypt in April 2011 and the plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Therese Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman garden, Giza, Egypt. A voucher specimen is deposited in the herbarium of the Agricultural Research Center, 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 Reagent Project. The cells were maintained at 37^{o}C in 5% CO_2 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 ($TCID_{50}$) in C8166 cells was determined and calculated as described by Reed and Muench **[9]**. 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 4×10^5 cells were plated into 96-well plates, 100 µl of various concentrations of compounds was added and incubated at 37^{o}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 **[10].**

Inhibition of syncytia formation

The effect of extracts on acute HIV-1 infectivity was measured by the syncytia formation assay **[11]**. In the presence or absence of various concentrations of samples, 4×10^4 C8166 cells were infected with HIV-1 at a multiplicity of infection (MOI) of 0.015, and cultured in 96-well plates at 37 ^{o}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 **[9]**, 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}

Preparation of the extracts

Finely ground aerial parts from *Carica papaya* 450 g were extracted with methanol and water/methanol (50:50) mixture by maceration. Each extract was concentrated to dryness to yield 35 of methanol extract and 28 g of aqueous 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 **[12-14]**.

III. RESULTS AND DISCUSSION

The results in **Table 1** showed that *Carica papaya* aerial parts polar extracts methanol and aqueous) were minimal toxic where methanol extract of *Carica papaya* was less toxic than aqueous extract, as well the extracts have drug ability as anti-HIV-1 agents where aqueous extract was more active than methanol as an anti-HIV-1 agents. Phytochemical analysis of *Carica papaya* polar extracts is shown in **table 4**. Cytotoxicity of the *Carica papaya* aerial parts extracts was carried out by using MTT colormetric methods. The results in **table 2** and **3** showed that *Carica papaya* extracts were minimal toxic and showed anti-HIV-1 activity. Methanol extract of *Carica papaya* had less cytotoxic effect; it was significantly different from that of the aqueous extract (**Table 1**). The anti-HIV-1 activity assay was performed by synctia formation. Aqueous extract of *carica papaya* aerial parts showed anti-HIV-1 activity and its therapeutic index (TI) value was the higher than that of methanol (**Table 2, Table 3**) with comparison with AZT. These results may be explained by the presence of phytochemicals in both methanol and aqueous extracts as triterpenes and/or sterols, tannins, flavonoids, carbohydrates and alkaloids (**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) **[15]**.

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 [15]. Tannins inhibit HIV-1 entry by targeting gp41 **[16]**, 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 **[17]**, as well luteolin cripples HIV-1 by abrogation of Tat function **[18]**, as well some phenolic compounds (flavonoids and tannins) have anti-HIV-1 activity **[19]**.

Table 1
Cytotoxicity of the extracts of *Carica papaya* in C8166 cell

The Extracts	Concentration (µg/ml)	Cell viability ±SD	CC_{50} (µg/ml)
Methanol	1000	-1.01 ± 1.35	337.006
	200	74.47 ± 1.13	
	40	116.67 ± 4.63	
	8	110.52 ± 7.42	
	1.6	104.31 ± 5.97	
	0.32	100.93 ± 4.50	
Aqueous	1000	20.76 ± 0.90	412.025
	200	73.84 ± 4.56	
	40	91.27 ± 1.54	
	8	91.22 ± 1.02	
	1.6	97.41 ± 1.66	
	0.32	94.86 ± 1.32	
AZT	4000	38.28 ± 0.86	1354.782
	800	86.71 ± 11.06	
	160	87.39 ± 1.77	
	32	88.60 ± 3.24	
	6.4	78.81 ± 2.57	
	1.28	80.42 ± 13.95	

Table 2
Anti-HIV activity of the extracts of *Carica papaya* in C8166 cell

The Extracts	Concentration (µg/ml)	Inhibition ± SD	EC_{50} (µg/ml)
Methanol	1000	100.00 ± 0.00	61.146
	200	100.00 ± 0.00	
	40	32.09 ± 7.26	
Aqueous	1000	100.00 ± 0.00	57.820
	200	96.93 ± 1.06	
	40	36.07 ± 8.61	
AZT	4000	98.13 ± 0.87	5.439
	800	93.58 ± 2.13	
	160	56.74 ± 3.56	
	32	28.62 ± 4.34	

Table 3
The summary of cytotoxicity and anti-HIV-1 activities of the extracts of *Carica papaya*

The Extracts	Method	CC_{50} (μg/ml)	EC_{50} (μg/ml)	Therapeutic index (TI)
Methanol	MTT	337.006	—	5.51
	CPE	—	61.146	
Aqueous	MTT	412.025	—	7.13
	CPE	—	57.82	
AZT	MTT	1354.782	—	249086.60
	CPE	—	5.439 ng/ml	

Table 4
Preliminary phytochemical screening of *Carica papaya* aerial parts extracts

Constituents	Methanol	Aqueous
Triterpenes and /or Sterols	+	+
Carbohydrates and/or glycosides	+	+
Flavonoids	+	+
Coumarins	-	-
Alkaloids and/or nitrogenous compounds	+	-
Tannins	+	+
Saponins	-	-
(+) presence of constituents, (-) absence of constituents		

IV. CONCLUSION

In this paper, *Carica papaya* aerial parts were extracted with methanol and 50% methanol (aqueous) solvents by maceration method and each extract was tested for its ability to act as anti-HIV-1 agent. Both extracts have drug ability to act as anti-HIV-1 agent where aqueous extract was is more active than methanol extract as an anti-HIV-1 agent and this may be explained by the presence of phytoconstituents as flavonoids, triterpenes, tannins, alkaloids and carbohydrates in the extracts and thus *Carica papaya* aerial parts polar extracts could provide chemical reservoir of anti-HIV-1 agents.

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CONFLICT OF INTEREST

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

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