International Journal of Molecular Biology & Biochemistry.
Volume 1, Number 1 (2013), pp. 1-7

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Inhibition of Human Immunodeficiency Virus (HIV-1) by *Hedera helix* L. Extracts and Phytoconstituents

Khaled Rashed[1*], Meng-Ting Luo[2], Lin-Tao Zhang[2] and Yong-Tang Zheng[2]

[1]National Research Centre, Pharmacognosy Department, Dokki, Giza, Egypt.
[2]Key Laboratory of Animal Models and Human Disease Mechanisms of Chinese Academy of Sciences and Yunnan province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China
[*]Corresponding authors: Dr. Khaled Nabih Zaki Rashed, E-mail: khalednabih2015@yahoo.co.uk

Abstract

This study deals with the evaluation of anti-HIV-1 effect of *Hedera helix* L. extracts and also the investigation of the phytoconstituents from the extracts of the plant. Chloroform, ethyl acetate and methanol 80% extracts of *Hedera helix* aerial parts were tested for their anti-HIV-1 activity using the syncytia formation assay. Methanol 80% *Hedera helix* aerial parts has shown anti-HIV-1activity and the other extracts were less active. Phytochemical profile of the plant extracts proves the presence of triterpenes, saponins, flavonoids, tannins and carbohydrates. The results have shown that *Hedera helix* methanol 80% extract has drug ability as anti- HIV-1 agent.

Keywords: *Hedera helix*, aerial parts, cytotoxicity, anti-HIV-1 activity, phytoconstituents.

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). *Hedera* is a genus of 15 species belong to the family Araliaceae, which has about 70 genera and 700 species of flowering plants**.** *Hedera helix* L. is an evergreen climbing plant, where suitable surfaces are available, and also growing as ground cover where there are no vertical surfaces. It is native to Ireland to Spain, Turkey and also Asia (2). In traditional medicine; the leaves were used as analgesic and anti-inflammatory agents. The leaves and berries were taken orally as an expectorant to treat cough and bronchitis. The leaves can cause severe contact dermatitis in some people. (3). Previous pharmacological studies proves that *H. helix* leaf has analgesic and antiinflmmatory activities (4), no report about phytocompounds from the plant. This study aimed to evaluate anti-HIV-1 effect of *Hedera helix* L. aerial parts extracts and also the investigation of the chemical content from the plant extracts.

Materials and methods

Plant Material

The aerial parts of *Hedera helix* were collected from Al-Zohiriya garden, Giza, Egypt in April 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 4×10^{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 (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, 4×10^4 C8166 cells were infected with HIV-1 at a multiplicity of infection (MOI) of 0.015, and cultured in 96-well plates at 37 ^oC 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 *Hedera helix* 400 g were extracted with chloroform, ethyl acetate and methanol 80% solvents by maceration. Each extract was concentrated to dryness to yield 15 g of chloroform, 8.5 g of ethyl acetate and 23 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 *Hedera helix* aerial parts extracts were minimal toxic where methanol 80% extract of *Hedera helix* was less toxic than the other two extracts. All the extracts have drug ability as anti-HIV-1 agents where methanol 80% extract was more active than chloroform and ethyl acetate extracts as an anti-HIV-1 agents, (Table 1, Table 2 and Table 3). Phytochemical analysis of *Hedera helix* aerial parts extracts is shown in table 4. The phytochemical analysis has shown the presence of triterpenes, saponins, flavonoids, tannins and carbohydrates. Cytotoxicity of the *Hedera helix* aerial parts extracts was carried out by using MTT colormetric methods, *Hedera helix* aerial parts extracts were minimal toxic and showed anti-HIV-1 activity. Methanol 80% extract of *Hedera helix* 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. Methanol 80% extract of *Hedera helix* 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 methanol extract 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). 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 *Hedera helix* in C8166 cell

Extracts	**Concentration (µg/ml)**	**Cell viability ±SD**	**CC_{50} (µg/ml)**
Chloroform	200	64.31 ± 3.57	>200
	40	100.1 ± 3.36	
	8	110.27 ± 4.52	
	1.6	103.44 ± 6.62	
	0.32	103.51 ± 6.93	
	0.064	93.94 ± 7.67	
Ethyl acetate	200	80.64 ± 1.47	>200
	40	129.65 ± 4.2	
	8	116.21 ± 1.16	
	1.6	109.38 ± 3.89	
	0.32	100.1 ± 3.36	
	0.064	88.14 ± 8.15	
Methanol 80%	200	1.49 ± 0.42	66.20
	40	72.1 ± 1.16	
	8	108.49 ± 0.95	
	1.6	94.9 ± 2.94	
	0.32	95.2 ± 5.46	
	0.064	98.39 ± 1.16	

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 *Hedera helix* in C8166 cell

Extracts	**Concentration (µg/ml)**	**Inhibition ± SD (%)**			**EC_{50} (µg/ml)**
Chloroform	200	100.00	±	0.00	91.49
	40	-2.89	±	3.51	
Ethyl acetate	200	84.5	±	4.38	101.45
	40	2.69	±	0.88	
Methanol 80%	200	100.00	±	0.00	26.49
	40	67.15	±	6.14	
	8	0.21	±	0.88	
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 the *Hedera helix* extracts

Extracts	**Method**	**CC_{50} (µg/ml)**	**EC_{50} (µg/ml)**	**Therapeutic index (TI)**
Chloroform	MTT	>200	—	>2.19
	CPE	—	91.49	
Ethyl acetate	MTT	>200	—	>1.97
	CPE	—	101.45	
Methanol 80%	MTT	66.2	—	2.5
	CPE	—	26.49	
AZT	MTT	1043µg/ml	—	459471
	CPE	—	2.27 ng/ml	

Table 4: Phytochemical Analysis from the *Hedera helix* 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

In this research paper, *Hedera helix* aerial parts were extracted 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 methanol 80% 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, alkaloids and carbohydrates in the methanol 80% extract and thus *Hedera helix* methanol 80% can act as anti-HIV-1 agent.

Acknowledgements

This work was supported in part by grants from 973 Program of China (2009CB522306), the Key Scientific and Technological Program of China (2012ZX10001-006) and NSFC (81001462, 81102483).

Conflict of interest

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

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