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EVALUATION OF ANTI–HIV–1 ACTIVITY OF *CORDIA MYXA* L. AND PHYTOCHEMICAL PROFILE

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Abstract. This study deals with evaluation of anti–HIV–1 activity from *Cordia myxa stems* extracts and investigation of phytochemical content of the plant extracts. Dichloromethane, ethyl acetate, and methanol 80% extracts of *Cordia myxa* were tested for their anti–HIV–1 activity using the syncytia formation assay. All the extracts showed a weak anti–HIV–1 activity. Phytochemical analysis of the extracts proved the presence of the following phytochemicals, triterpenes in dichloromethane extract. Flavonoids and triterpenes in ethyl acetate extract. Carbohydrates, flavonoids, and triterpenes in methanol extract. This research work gave a brief note about main chemical constituents and anti–HIV–1–activity of *Cordia myxa* extracts.

Key words: *Cordia myxa*, stems, cytotoxicity, anti–HIV–1 activity, Phytoconstituents.

Introduction

The human Immunodeficiency virus type 1 (HIV–1) is an aetiological agent for Acquired Immunodeficiency Syndrome (AIDS). HIV–1 is cause of 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 [MLINARIC *et al.*, 2000].

Cordia myxa, a perennial generative and vegetative plant, from family *Boraginaceae* is popularly used by public for its efficacy in chest and urinary infections.

Other known therapeutic properties of plant are anthelmintic, diuretic, demulcent, antidiarrheal, anti–gastric and anti–worm have been reported, also it is a liver tonic [AL–AWADI *et al*. 2001].

Several preparations of *Cordia* species from its bark, leaf and fruit extracts have been used in traditional medicine for treatment of osteoarticular diseases, dysmenorrhea and as abortive [AFZAL *et al*., 2009].

Analgesic, anti–inflammatory and anti–arthritic activities of them have been studied in rats [FICARRA *et al*., 1995; AL–AWADI *et al*. 2001].

This study evaluated anti–HIV–1 activity and phytoconstituents from *Cordia myxa* stems extracts.

Materials and methods

Plant Material

The stems of *Cordia myxa* were collected from Al–Zohiriya garden, Giza, Egypt in May 2013.

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 Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen is deposited in 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°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°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 (TCID50) in C8166 cells was determined and calculated by Reed and Muench (1938) [REED and MUENCH, 1938].

Virus stocks were stored in small aliquots at –70°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°C in a humidified atmosphere of 5% CO_2 for 72h.

100 μL of supernatant was discarded; MTT reagent was added and incubated for 4 h and 100 μL 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 [WANG *et al.*, 2008, BUTNARIU and CAUNII, 2013].

Inhibition of syncytia formation

The effect of extracts on acute HIV–1 infectivity was measured by syncytia formation assay [HUANG *et al.*, 2012, RASHED and BUTNARIU, 2012].

In 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°C in 5% CO_2 for 3 days.

AZT was used as a positive control.

At 3 days post–infection, cytopathic effect (CPE) was measured by counting 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 percentage of syncytia number in sample–treated culture compared to that in infected control culture 50% effective concentration (EC_{50}) was calculated according to method described by Reed and Muench [REED and MUENCH, 1938, SAMFIRA *et al.*, 2013], 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}

$$\text{Cell viability (\%control)}=(OD_{test}-OD_{blk})/(OD_{ctrl}-OD_{blk})\times100$$

$$\text{CPE inhibition(\%)}=(1-CPE_{test}/CPE_{ctrl})\times100$$

Preparation of the extracts

Finely ground stems from *Cordia myxa* (250) g were extracted with dichloromethnae, ethyl acetate, and methanol 80% solvents by maceration.

Each extract was concentrated to dryness to yield 9.5 g of chloroform, 6.5 g of ethyl acetate, and 14.5 g of methanol 80% extract.

Each extract was tested for presence of the phytoconstituents according to 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

[SOFOWRA, 1993; TREASE, and EVANS, 1989; HARBORNE, 1973, RASHED and BUTNARIU, 2014].

Results and Discussion

The results showed that *Cordia myxa* stems extracts were minimal toxic where ethyl acetate extract of *Cordia myxa* was less toxic than other two extracts.

All extracts have weak drug ability as anti–HIV–1 agents, where chloroform extract was more active than ethyl acetate, and methanol 80% extracts as an anti–HIV–1 agent (Table 1, Table 2 and Table 3).

The phytochemical analysis has shown presence of triterpenes, flavonoids, tannins and carbohydrates in methanol extract.

Table 1.

Cytotoxicity of the extracts of *Cordia myxa* stems in C8166 cell

Extracts	Concentration (µg/mL)	Cell viability±SD(%)	CC_{50} (µg/mL)
Dichloromethane	200	91.74±3.99	>200
	40	106.78±3.94	
	8	104.28±10.05	
	1.6	105.93±2.48	
	0.32	104.57±5.88	
	0.064	100.44±5.17	
Ethyl acetate	1000	6.23±0.75	120.101
	200	23.99±0.60	
	40	106.08±6.29	
	8	95.33±2.84	
	1.6	105.89±2.54	
	0.32	101.39±4.69	
Methanol 80%	1000	55.25±0.71	>1000
	200	82.42±1.48	
	40	89.20±2.41	
	8	96.08±4.68	
	1.6	101.71±2.54	
	0.32	97.43±8.45	
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	

Cytotoxicity of *Cordia myxa* stems extracts was carried out by using MTT colormetric methods, *Cordia myxa* stems extracts were minimal toxic and showed a weak anti–HIV–1 activity.

Table 2.

Anti–HIV activity of the extracts of *Cordia myxa* stems in C8166 cell

Extracts	Concentration (µg/mL)	Inhibition±SD(%)	EC_{50} (µg/mL)
Dichloromethane	200	95.44±3.25	62.336
	40	32.71±7.25	
Ethyl acetate	1000	100.00±0.00	68.197
	200	100.00±0.00	
	40	25.21±6.52	
Methanol 80%	1000	100.00±0.00	334.025
	200	26.61±10.26	
AZT	4000	98.13±0.87	5.439
	800	93.58±2.13	
	160	56.74±3.56	
	32	28.62±4.34	

Dichloromethane extract of *Cordia myxa* stems had less cytotoxic effect; it was significantly different from that of the other two extracts (Table 1).

Anti–HIV–1 activity assay was performed by syfunctia formation.

All extracts of *Cordia myxa* stems showed a weak anti–HIV–1 activity and its therapeutic index (TI) value was than 10 (table 2, table 3) with comparison with AZT.

Table 3.

The summary of cytotoxicity and anti–HIV–1 activities of the extracts of *Cordia myxa* stems

Extracts	Experiment	Method	CC_{50}(μg/mL)	EC_{50}(μg/mL)	Therapeutic index (TI)
Dichloro methane	Cytotoxicity assay	MTT	>200	—	>3.21
	Inhibition of syncytium formation	CPE	—	62.336	
Ethyl acetate	Cytotoxicity assay	MTT	120.101	—	1.76
	Inhibition of syncytium formation	CPE	—	68.197	
Methanol 80%	Cytotoxicity assay	MTT	>1000	—	>2.99
	Inhibition of syncytium formation	CPE	—	334.025	
AZT	Cytotoxicity assay	MTT	1354.782	—	249086.60
	Inhibition of syncytium formation	CPE	—	5.439 ng/mL	

Dichloromethane extract proved presence of triterpenes while ethyl acetate extract contained triterpenes, and flavonoids (table 4).

Dichloromethane extract was more active than ethyl acetate and methanol extracts, it showed therapeutic index (TI) >3.21.

The extracts of *Cordia myxa* stems have interesting bio–active constituents, as triterpenes, tannins, flavonoids and carbohydrates.

Table 4.

Phytochemical Analysis of *Cordia myxa* stems extracts

Constituents	Dichloromethane	Ethyl acetate	Methanol 80%
Triterpenes and /or Sterols	+	+	+
Carbohydrates and/or glycosides	–	–	+
Flavonoids	–	+	+
Coumarins	–	–	–
Alkaloids and/or nitrogenous compounds	–	–	–
Tannins	–	–	+
Saponins	–	–	–

(+) presence of the constituents, (–) absence of the constituents

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 EC_{50} 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 [KASHIWADA *et al.*, 1998, BUTU *et al.*, 2014].

Tannins inhibit HIV–1 entry by targeting gp41 [COLLINS *et al.*, 1997, BOSTAN et al., 2013], 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 viral proteins that mediate late steps of HIV replication [LU *et al.*, 2004], as well luteolin cripples HIV–1 by abrogation of tat function [RAJEEV *et al.*, 2011, BUTNARIU and BOSTAN, 2011], as well some phenolic compounds (flavonoids and tannins) have anti–HIV–1 activity [RASHED *et al.*, 2012, BUTNARIU, 2014].

Although the presence of these active principles, there is a weak anti–

HIV–1 activity and this is may be due synergistic effect.

Conclusion

We extracted *Cordia myxa* stems extracts with dichloromethane, 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 weak drug ability to act as anti–HIV–1 agent.

Dichloromethane extract was more active than ethyl acetate and methanol extracts, it showed therapeutic index (TI) >3.21.

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