The Journal of Microbiology (2010) Vol. 48, No. 2, pp. 249-252
DOI 10.1007/s12275-009-0176-9

NOTE

Anti-HIV-1 Efficacy of Extracts from Medicinal Plants

Su-A Lee[1], Seong-Karp Hong[1], Chang-Il Suh[1], Mi-Hwa Oh[1], Jeong-Ho Park[2], Byoung-Wook Choi[2], Seung-Won Park[1], and Soon-Young Paik[1]*

[1]*Department of Microbiology, College of Medicine, The Catholic University of Korea, Seoul 137-701, Republic of Korea*
[2]*Department of Applied Chemistry and Biotechnology, Hanbat National University, Daejeon 305-719, Republic of Korea*

(Received June 5, 2009 / Accepted September 28, 2009)

The anti-HIV-1 activities of butanol, hexane, chloroform and water extracts from four widely used folk medicinal plants (*Sophora flavescens*, *Tulipa edulis*, *Herba ephedra*, and *Pachyma hoelen Rumph*) were evaluated in this study. The hexane extract of *Pachyma hoelen Rumph*, PH-4, showed effective inhibition against HIV-1. The 50% effective concentration (EC_{50}) of PH-4 was 37.3 μg/ml in the p24 antigen assay and 36.8% in the HIV-1 recombinant RT activity test (at 200 μg/ml). In addition, the PH-4 showed the protective effect on the infected MT-4 cells, with a 58.2% rate of protection. The 50% cytotoxic concentration (CC_{50}) of PH-4 was 100.6 μg/ml. These results suggest that PH-4 from *Pachyma hoelen Rumph* might be the candidate for the chemotherapy agent against HIV-1 infection with further study.

***Keywords*:** Anti-HIV-1, aqueous extracts, folk medicine, medicinal plants, organic extracts

With the identification of the retrovirus, human immunodeficiency virus type 1 (HIV-1) (Barre-Sinoussi *et al.,* 1983; Gallo *et al*., 1983) as the causative agent of acquired immunodeficiency syndrome (AIDS), a number of laboratories have been actively involved in the development of antiviral agents that interfere with HIV at different stages of viral replication (Balzarini *et al*., 1986; Wilkinson *et al*., 1999; Clavel and Hance, 2004). Unfortunately, treatment of AIDS patients is limited by the emergence of resistant virus, cross-resistance to drugs and cell toxicity (Balzarini *et al*., 1986; Tantillo *et al*., 1994; Lipsky, 1996).

Over the past 20 years, progress has been made in screening and developing natural products and chemically synthesized compounds as medication for HIV infections (Vlietinck *et al*., 1998; De Clercq, 2000; Kong *et al.*, 2003; Wang *et al*., 2004, 2008; Hupfeld and Efferth, 2009). Medicinal plants are the source of many medications and might provide alternative therapies for HIV in developing countries that are at the center of the AIDS pandemic (Ito *et al*., 1988; Lederman *et al*., 2006; Klos *et al*., 2009). In Korea, several medicinal plant products or traditional medicines have been prescribed to treat patients with AIDS. Some have claimed that certain medicinal plant remedies improve the quality of life of patients with AIDS by reducing the viral load (Ahn *et al*., 2002). However, the efficacy of such remedies and plants requires confirmation.

This research was carried out to assess the effect of selected Korean medicinal plants thought to inhibit HIV-1 (Patwardhan and Gautam, 2005). In this study, four sets of organic and aqueous extracts of four Korean medicinal plants were studied for anit-HIV-1 activity. Since some of the plant extracts significantly inhibited the enzyme activity of HIV-1 replication and protected cells infected with HIV-1, the extracts studied are candidates for further study and the development of potent inhibitors of HIV-1 that might be useful in the clinical setting.

The oriental medicine and medicinal plants used in this study were obtained from OBM Laboratories, LTD, Daejeon, South Korea, and identified by Professor Seok-Sun Roh at Daejeon University. The vouchers for the species (HNU-03011∼HNU-03014) have been deposited at Laboratory of Natural Products Chemistry, Hanbat National University.

Each sample (100 g) was extracted with methanol at room temperature (3×1 L, 24 h for each time). The extract was concentrated under reduced pressure and the residue was partitioned between hexane (500 ml) and 80% methanol (500 ml). The 80% methanol layer was concentrated under reduced pressure and further partitioned between 30% methanol (500 ml) and chloroform (500 ml). The 30% methanol layer was concentrated under reduced pressure and partitioned again between butanol (500 ml) and H_2O (500 ml). Each solvent fraction was obtained from evaporation in a vacuum and the extracts from each procedure were named as SF1-4, TE1-4, HE1-4, and PH1-4 (Table 1).

The cell toxicity of the extracts was assessed by WST-1 methods as described previously (Balzarini *et al*., 1986) using MT4 cells cultured in RPMI-1640 (supplemented with 10% heat inactivated newborn calf serum, Gibco, USA). The cells were incubated with 0.5 mM of WST-1 solution containing 20 mM of 1-methoxy PMS (Dojindo, Japan) at 37°C for 2 h. The production of WST-1 formazan was measured by a microplate

* For correspondence. E-mail: paik@catholic.ac.kr; Tel: +82-2-590-1225; Fax: +82-2-590-1225

Table 1. Summaries of plant extract on cytotoxicities and anti-HIV-1 activities

Plant name	Fraction and sample		Cytotoxicity (CC_{50}, μg/ml)	p24 antigen (EC_{50}, μg/ml)	RT activity (%, 200 μg/ml)	Protection (%, 40 μg/ml)
Sophora flavescens	H_2O	SF-1	>200	>200	NI	21.7
	Butanol	SF-2	>200	64.6	NI	35.5
	$CHCl_3$	SF-3	>200	5.8	NI	13.5
	Hexane	SF-4	70.7	158.9	12.9	24.9
Tulipa edulis	H_2O	TE-1	>200	>200	19.5	22.9
	Butanol	TE-2	>200	75.6	NI	43.9
	$CHCl_3$	TE-3	>200	93.7	NI	13.3
	Hexane	TE-4	38.3	160.1	11.6	27.7
Herba ephedra	H_2O	HE-1	163.2	>200	NI	27.1
	Butanol	HE-2	120.8	138.6	NI	75.6
	$CHCl_3$	HE-3	151.1	29.9	NI	72.4
	Hexane	HE-4	68.8	161.1	12.0	80.0
Pachyma hoelen Rumph	H_2O	PH-1	>200	>200	NI	40.9
	Butanol	PH-2	142.5	135.4	NI	73.0
	$CHCl_3$	PH-3	>200	84.9	NI	32.8
	Hexane	PH-4	100.6	37.3	36.8	58.2

NI, no inhibition. Values in parenthesis are given in μg/ml. Data was collected from three independent experiments and expressed as means.

reader at 450 nm and the cytotoxicity of the inhibitors was calculated as the relative rate of WST-1 formazan production to that of the dox-treated cells (Mosmann, 1983). The cytotoxic concentration that caused the reduction of viable cells by 50% (CC_{50}) was calculated from the dose-response curve (Table 1).

The effect of the extracts on HIV-1 replication *in vitro* was measured by a p24 release assay (Vironostika, BioMerieux Co., Netherlands) (Zheng *et al*., 2000). The wells of the micro elisa strips were coated with antibodies to the HIV-1 p24 core antigen. Disruption buffer was added to disrupt the HIV-1 virions present in the test samples. All samples (100 μl in each well) were incubated at 37°C for 60 min. After washing with phosphate-buffered saline (PBS), 100 μl of anti-HIV-1 (human) conjugate labeled with horseradish peroxidase (HRP) was added to each well, and incubated at 37°C for 60 min. Then 100 μl of tetramethylbenzidine substrate in urea peroxide solution was added to each well after a washing step, and the plates were incubated at room temperature for 30 min. Finally, the color reaction was stopped by adding 100 μl of 1 M sulfuric acid. The absorbance of each well was read at 450 nm within 15 min by the ELISA reader. The concentration of reducing p24 antigen expression by 50% (EC_{50}) was determined from the dose-response curve. The EC_{50} by the P24 antigen assay of SF-3, HE-3, and PH-4 had a higher inhibition of HIV-1 than the other extracts and were 5.8, 29.9, and 37.3, respectively (Table 1).

The effect of the crude extracts on HIV-1 reverse transcription was tested using a non-radioactive HIV-RT colorimetric ELISA kit (Roche Diagnostics, Germany) (Goncalves *et al*., 1996; Ahn *et al*., 2002, 2004). The protocol outlined in the kit was followed using 2 ng of enzyme in a well and incubating the reaction for 2 h at 37°C. The extracts were tested at 0.2 mg/ml (Wang *et al*., 2006a, 2006b). Most extracts did not inhibit the activity of recombinant HIV-1 RT. SF-4, TE-1, TE-4, HE-4, and PH-4 showed inhibition of the activity of recombinant HIV-1 RT as 12.9, 19.5, 11.6, 12.0, and 36.8% at a concentration of 200 μg/ml (Table 1).

The activity of the extracts against acute HIV-1 infection was based on the inhibition of HIV-1 induced cytopathogenicity in the MT-4 cells as described previously (Sarin, 1988). Mock infected (uninfected) or HIV-1IIIB (obtained from the culture supernatant of H9/HIV-1_{IIIB} cell) infected (MOI=0.1) MT-4 cells (4×10^5 cells/ml) were seeded in 96-well flat-bottomed microtiter culture plates with 100 μl of different concentrations of the extracts. Antiretroviral agent inhibiting reverse transcriptase, R-9-2-phosphonomethoxypropyl adenine (PMPA) (provided by the NIH AIDS Research and Reference Reagent Program, USA) and the approved HIV drug inhibiting reverse transcriptase, 3'-azido-3'-deoxythymidine (AZT) (Sigma-Aldrich, USA) were used for control drugs. After 7 days of incubation at 37°C, the viability of both the HIV-1- and mock-infected cells was assessed by WST-1 methods. The data on the Fig. 1 are expressed as a percentage of untreated uninfected control cells (Wang *et al*., 2006a). Among all extracts tested for the protection of cells infected with HIV-1, HE-2, HE-3, HE-4, and PH-2 exhibited a high protection against HIV-1 lytic effects on the cells *in vitro* (Table 1). At concentrations 40 μg/ml, the protection of infected cells was 75.6, 72.4, 80.0, and 73.0%, respectively.

Recently, evidence based analysis of natural plants used in traditional medicine has been of great interest. Generally, medicinal plants include a broad number of species with complex composition. Some have shown that natural plants might be more safe and effective than random drug-screening (Patwardhan and Gautam, 2005). The biological and pharmacological benefits of the broad range of plant characteristics

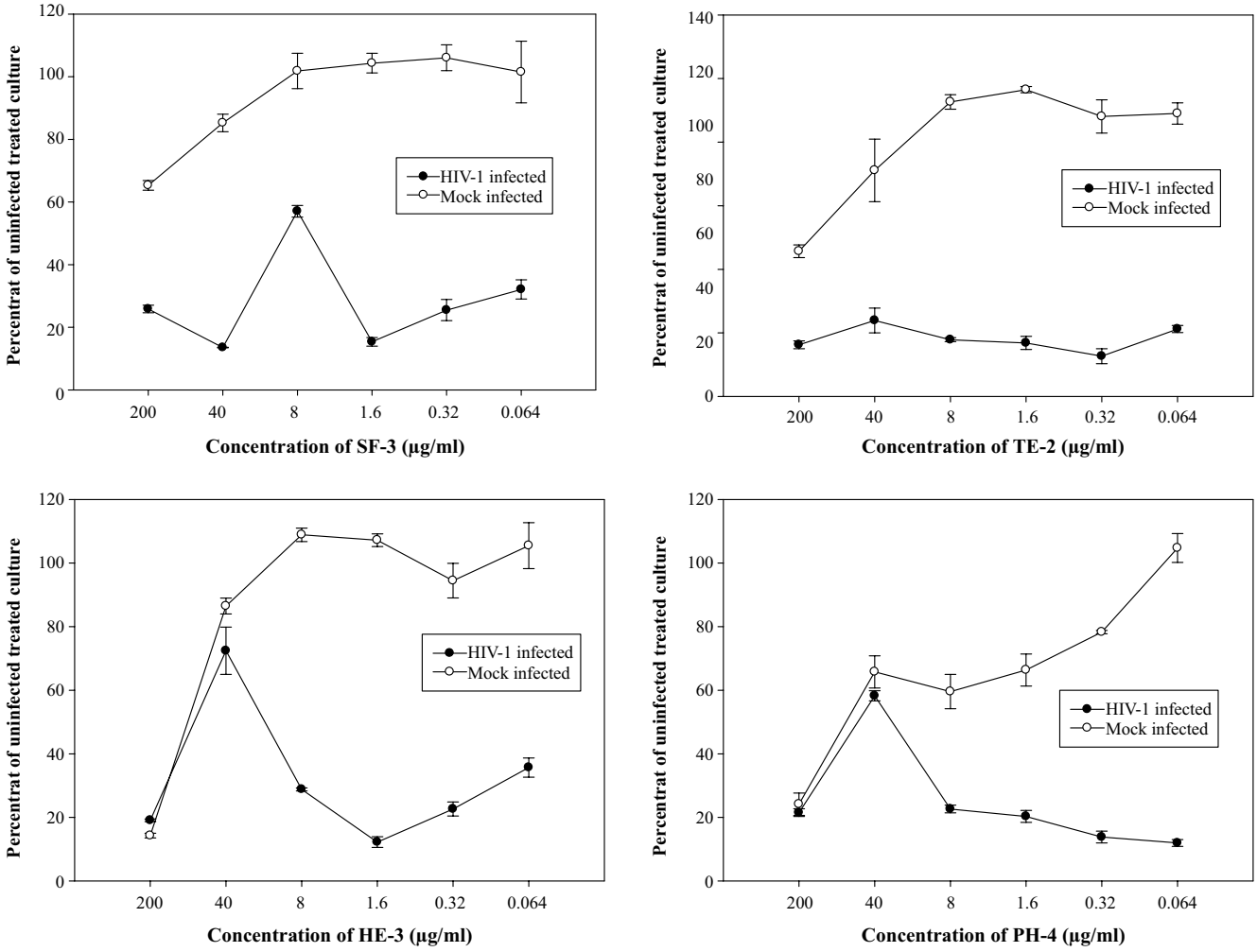

Fig. 1. The protection effects of four representative fractions on HIV-1 induced lytic effects. (A) SF-3; (B) TE-2; (C) HE-3; (D) PH-4. The data are collected from three independent experiments and expressed as percentages (±SD) of uninfected controls.

have been shown to have effective activity against HIV-1 (Bedoya *et al*., 2001).

In this study, the anti-HIV-1 activity of butanol, hexane, chloroform, and water extracts from four medicinal plants, widely used in the folk medicine, were evaluated. Among these extracts, some showed anti-HIV-1 activity by inhibition tests against HIV-1. A chloroform extract, HE-3, of *Herba ephedra* also had good anti-HIV-1 activity. HE-3 had a 29.9 μg/ml (EC_{50}) on the p24 antigen assay. In addition, HE-3 had protective effects on the infected MT-4 cells; the protection was 72.4%. The CC_{50} of HE-3 was 151.1 μg/ml. But it has no inhibition activity on the RT activity test (at 200 μg/ml). A henxane extract, HE-4, of had relatively low antiviral activity, but had the highest score from the protection test, 80%.

In addition, other extracts also showed anti-HIV-1 activity. The chloroform extract, SF-3, of *Sophora flavescens* had the best anti-HIV-1 activity; 5.8 μg/ml in the p24 antigen assay and a high concentration >200 μg/ml in the CC_{50}. However, it did not inhibit HIV-1 by RT activity and had low inhibition on the protection test.

Compared to other plant extracts, TE samples from *Tulipa edulis* did not show good anti-HIV-1 effects on the p24 antigen assay and protection test. Among them, only TE-2 showed some effects on the inhibition of p24 antigen production and on the protection of infected cells.

A hexane extract, PH-4, of *Pachyma hoelen Rumph* was shown to have the best anti-HIV-1 activity compared to the other extracts. PH-4 had 37.3 μg/ml (EC_{50}) on the p24 antigen assay and the highest value, 36.8% on the RT activity test (at 200 μg/ml). In addition, PH-4 protected the infected MT-4 cells; the protection was the highest observed at 58.2%. The CC_{50} of PH-4 was 100.6 μg/ml. Therefore, PH-4 was the most effective against HIV-1 among all extracts studied.

The results of this study suggest that the PH-4 is most effective at viral entry, replication, and after the reverse transcription of the HIV-1 life cycle. In particular, the early steps of life cycle of HIV-1 is likely to be target of its action as shown on Table 1 data as well as the action mode of protection of lytic effects shown on Fig. 1 pattern. In conclusion, PH-4, a hexane extract, of *Pachyma hoelen Rumph* was shown to have significant activity against HIV-1. And future studies are needed to find out effective molecule from PH-4 fraction and

to figure out how it works for the new AIDS drug design.

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2006-E00010), the BK21 project team for Biomedical Science, and Waterborne Virus Bank funded by National Research Foundation of Korea.

References

Ahn, M.J., K.D. Yoon, and C.Y. Kim. 2002. Inhibition of HIV-1 reverse transcriptase and HIV-1 integrase and antiviral activity of Korean seaweed extracts. *J. Appl. Phycol.* 14, 325-329.

Ahn, M.J., K.D. Yoon, and S.Y. Min. 2004. Inhibition of HIV-1 reverse transcriptase and protease by phlorotannins from the brown alga, *Ecklonia cava*. *Biol. Pharm. Bull.* 27, 544-547.

Balzarini, J., H. Mitsuya, E. De Clercq, and S. Broder. 1986. Comparative inhibitory effects of suramin and other selected compounds on the infectivity and replication of human T-cell lymphotropic virus (HTLV-III)/lymphoadenopathy-associated virus (LAV). *Int. J. Cancer* 37, 451-457.

Barre-Sinoussi, F., J.C. Chermann, F. Rey, M.T. Nugeyre, S. Chamaret, C. Gruest, C. Dauguest, and *et al*. 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 220, 868-871.

Bedoya, L.M., S. Sanchez-Palomino, M.J. Abad, P. Bermejo, and J. Alcami. 2001. Anti-HIV activity of medicinal plant extracts. *J. Ethnopharmcol.* 77, 113-116.

Clavel, F. and A.J. Hance. 2004. HIV drug resistance. *N. Engl. J. Med*. 350, 1023-1035.

De Clercq, E. 2000. Current lead natural products for the chemotherapy of human immuno deficiency virus infection. *Med. Res. Rev.* 20, 323-349.

Gallo, R.C., P.S. Sarin, E.P. Gelmann, M. Robert-Guroff, E. Richardson, V.S. Kalyanaraman, D. Mann, and *et al*. 1983. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). *Science* 220, 865-867.

Goncalves, J., Y. Korin, J. Zack, and D. Gabuzda. 1996. Role of Vif in human immunodeficiency virus type 1 reverse transcription. *J. Virol*. 70, 8701-8709.

Hupfeld, J. and T. Efferth. 2009. Drug resistance of human immunodeficiency virus and overcoming it by natural products. *In Vivo* 23, 1-6.

Ito, M., A. Sato, K. Hirabayashi, F. Tanabe, S. Shigeta, M. Baba, E. De Clercq, H. Nakashima, and N. Yamamoto. 1988. Mechanism of inhibitory effect of glycyrrhizin on replication human immunodeficiency virus (HIV). *Antiviral Res.* 10, 289-298.

Klos, M., M. van de Venter, P.J. Milne, H.N. Traore, D. Meyer, and V. Oosthuizen. 2009. *In vitro* anti-HIV activity of five selected South African medicinal plant extracts. *J. Ethnopharmacol.* 124, 182-188.

Kong, J.M., N.K. Goh, L.S. Chia, and T.F. Chia. 2003. Recent advances in traditional plant drugs and orchids. *Acta Pharmacol. Sin.* 24, 7-21.

Lederman, M.M., R.E. Offord, and O. Hartley. 2006. Microbicides and other topical strategies to prevent vaginal transmission of HIV. *Nat. Rev. Immunol*. 6, 371-382.

Lipsky, J.J. 1996. Antiretroviral drugs for AIDS. *Lancet* 348, 800-803.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65, 55-63.

Patwardhan, B. and M. Gautam. 2005. Botanical immunodrugs: scope and opportunities. *Drug Discovery Today* 10, 495-502.

Sarin, P.S. 1988. Molecular pharmacologic approaches to the treatment AIDS. *Annu. Rev. Pharmacol. Toxicol.* 28, 411-428.

Tantillo, C., J. Ding, A. Jacobo-Molina, R.G. Nanni, P.L. Boyer, S.H. Hughes, R. Pauwels, K. Andries, P.A.J. Janssen, and E. Arnold. 1994. Locations of anti-AIDS drug binding sites and resistance mutations in the three-dimensional structure of HIV-1 reverse transcriptase. Implications for mechanisms of drug inhibition and resistance. *J. Mol. Biol.* 243, 369-387.

Vlietinck, A.J., T. De Bruyne, S. Apers, and L.A. Pieters. 1998. Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection. *Planta. Med.* 64, 97-109.

Wang, Q., Z.H. Ding, J.K. Liu, and Y.T. Zheng. 2004. Xanthohumol, a novel anti-HIV-1 agent purified from Hops *Humulus lupulus*. *Antiviral Res.* 64, 189-194.

Wang, R.R., Q. Gu, Y.H. Wang, X.M. Zhang, L.M. Yang, J. Zhou, J.J. Chen, and Y.T. Zheng. 2008. Anti-HIV-1 activities of compounds isolated from the medicinal plant *Rhus chinensis*. *J. Ethnopharmacol.* 17, 249-256.

Wang, R.R., Q. Wang, L.M. Gu, J.J. Yang, S. Chen, Y. Li, and Y.T. Zheng. 2006a. Anti-HIV-1 activities of extracts from the medicinal plant *Rhus chinensis*. *J. Ethnopharmacol.* 105, 269-273.

Wang, Q., R.R. Wang, L.M. Yang, W. Li, M. Xu, and Y.T. Zheng. 2006b. Expression, purification of HIV-1 protease and the establishment of a method for protease inhibitor screening. *Virologica Sinica* 21, 126-130.

Wilkinson, D., L. Gcabashe, and M. Lurie. 1999. Traditional healers as tuberculosis treatment supervisors: precedent and potential. *Int. J. Tuberc. Lung Dis.* 3, 838-842.

Zheng, Y.T., K.L. Zheng, L. Ben, and S.W. Jin. 2000. Anti-HIV-1 activity of trichosanthin, a novel ribosome-inactivating protein. *Acta Pharmacol. Sin.* 21, 179-182.