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Inhibitory Activities of Korean Plants on HIV-1 Protease

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Abstract – For the development of anti-AIDS agents, thirty-seven methanol extracts of Korean plant materials were tested for their inhibitory effects on human immunodeficiency virus type-1 (HIV-1) protease. Extracts of seven plants showed more than 30% inhibitory activities on HIV-1 protease at a concentration of 100 μg/ml. The bark of *Berchemia berchemiaefolia*, the leaf of *Lindera erythrocarpa* and the whole plant of *Siegesbeckia pubescens* exhibited significant inhibititory activities on HIV-1 protease with 56.2, 50.8, and 46.6%, respectively.

Key words – acquired immunodeficiency syndrome, human immunodeficiency virus type-1, HIV-1 protease, protease inhibitors, Korean plants, *Berchemia berchemiaefolia*.

Introduction

Human immunodeficiency virus type 1 (HIV-1) has been given consideration to be the etiological agents of acquired immunodeficiency syndrome (AIDS) (Barre-Sinoussi *et al.*, 1983). The search for anti-HIV drugs has been a continuous attempt to obtain therapy for AIDS. For clinical use in the treatment of AIDS, nucleoside analogs, such as zidovudine (3'-azido-2',3'-dideoxythymidine, azidothymidine, AZT), didanosine (2',3'-dideoxyinosine, DDI), zalcitabine (2',3'-dideoxycytidine, DDC), and stavudine (2',3'-didehydro-3'-deoxythymidine, d4T), are known to show the development of resistant virus and undesirable side effects in long-term use (Larder *et al.*, 1989). The effective drugs with reduced toxicity has been required for treatment of AIDS. Most of development for anti-AIDS agents are based on the blocking steps of viral life cycle, such as adsorption of the virus particle to the host cell, synthesis of viral DNA by reverse transcriptase, viral proteolytic process by protease and synthesis of viral envelope glycoproteins (De Clercq, 1990). A protease of HIV-1 has been demonstrated to play an essential function in viral replication (Dark *et al.*, 1988). Genetic and biochemical studies have demonstrated that the polyproteins are proteolytically processed by the action of a virus-encoded protease. Therefore, the inhibition of HIV-1 protease has been become a promising target for the development of anti-viral agents for AIDS (Ido *et al.*, 1991). For the purpose of finding specific inhibitors of HIV-1 protease, we screened thirty-seven methanol extracts of Korean plants. As the results, some extracts exhibited inhibitory effects and were found to be candidated for further study on the active principles.

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Experimental

Materials – All plants were collected at Republic of Korea and identified by Prof. KiHwan Bae of Chungnam National University, Korea. The voucher specimens are deposited at the herbarium of College of Pharmacy, Chungnam National University.

Chemicals – The fused recombinant HIV-1 protease was obtained as reported before (Kusmoto *et al.*, 1995). The substrate His-Lys-Ala-Arg-Val-Leu-(pNO_2-Phe)-Glu-Ala-Nle-Ser-NH_2, a modified amino acid sequence of p24-p15 cleavage site of the viral polyprotein, was purchased Peptide Institute, Inc. (Osaka, Japan). Acetyl pepstatin was purchased from BACHEM Feinchemikalien AG (Bubendorf, Switzerland).

Preparation of plants extracts – Twenty grams of each dried plant samples were extracted with 100 ml MeOH under room temperature. The extracts were filtered and dried *in vacuo*. For the test, the MeOH extracts were dissolved in dimethyl sulfoxide (DMSO) and final concentration of DMSO was adjusted below 10%.

HIV-1 PR assay – A reaction mixture (5 μL) containing 50 mM acetate buffer (pH 5.0), 1.5 mM of substrate, 1 μL of a plant extract and 3.4 μM recombinant HIV-1 PR solution were added and the mixture was incubated at 37°C for 120 min. A control reaction was performed under the same conditions without the addition of plant extracts. The reaction was stopped by heating the mixture at 90°C for 1 min. Then, 35 μL of water was added and an aliquot of 5 μL was analyzed by HPLC. The HPLC system consisted of an LC9A liquid chromatography, SPD-6A UV spectrophotometeric detector, SLC-6B autoinjector and an integrator C-R 6A Chromatopac (Shimadzu Corporation, Kyoto, Japan) was used for analysis of protease hydrolysates. Five microliters of the reaction mixture was injected into a RP-18 column (4.6×150 mm, YMC Co., Kyoto, Japan), eluted with a gradient of acetonitrile (15-35%) in 0.1% TFA at a flow of 1.0 mL/min. The elution profile was monitored at 280 nm. The substrate and pNO_2-Phe-bearing hydrolysate were eluted at 11.55 and 5.05 min, respectively. The protease activity was calculated from the ratio of the substrate peak area to the product peak area. Acetyl pepstatin, which showed 50% inhibitory activity (IC_{50}) at 29 μg/ml, was used as a positive control of inhibition (Richard *et al.*, 1989).

Results and Discussion

In the course of searching for naturally-occurring substance with anti-AIDS agents, we had screened natural products used in traditional medicines in China, India, Indonesia, Sri Lanka, Panama and Egypt for their inhibitory effects on HIV-1 specific enzymes, such as reverse transcriptase and protease, and also on the replication of HIV-1. We had isolated the HIV-1 protease inhibitory substance from *Areca catechu*, *Swietenia mahagoni* and *Ganoderma lucidum* and reported that isolated compounds showed for the inhibitory effects of HIV-1 protease (Kusumoto *et al.*, 1995, Matsuse *et al.*, 1997). The present trial is a preliminary test of Korean plants for inhibitory effect on HIV-1 protease. Among the thirty-seven MeOH extracts of Korean plants, seven plants, such as the root of *Acanthopanax koreanum*, the whole plant of *Aruncus dioicus* var. *kamtschaticus*, the bark of *Berchemia berchemiaefolia*, the stem of *Crataegus pinnatifida*, the leaf of *Lindera erythrocarpa*, the whole plant of *Siegesbeckia pubescens* and the leaf of *Tilia amurensis*, were found inhibitory activities (32.1-56.2%) of the recombinant enzyme at a concentration of 100 μg/ml (Table 1). At a test concentration, *B. berchemiaefolia* showed the most inhibitory effect on HIV-1 protease with inhibition of 56.2%. On the chem-

Table 1. HIV-1 protease inhibitory activities of MeOH extracts of Korean plants

Botanical name	Family	Part used	Inhibition (%)[1)]
Acanthopanax chiisanensis NAKAI	Araliaceae	Seed	0.6±6.5
Acanthopanax koreanum NAKAI	Araliaceae	Root	38.7±6.5
Actinidia arguta PLANCH.	Actinidiaceae	Leaf, Stem	N. E.[2)]
Anemarrhena asphodeloides BUNGE	Haemodoraceae	Root	N. E.
Artemisia iwayomogi KITAMURA	Compositae	Root	N. E.
Aruncus dioicus var. *kamtschaticus* HARA	Rosaceae	Whole plant	32.1±8.6
Aster koraiensis NAKAI	Compositae	Leaf	16.8±5.1
Berchemia berchemiaefolia KOIDZ.	Rhamnaceae	Bark	56.2±2.5
Bupleurum longiradiatum TURCZ.	Umbellifeae	Root	6.3±5.9
Campsis grandiflora K. SCHUM.	Bignoniaceae	Leaf, Stem	N. E.
Campsis grandiflora K. SCHUM.	Bignoniaceae	Root	N. E.
Carpesium abrotanoides L.	Compositae	Whole plant	4.2±2.5
Clematis apiifolia A. P. DC.	Ranunculaceae	Whole plant	N. E.
Clematis heracleifolia DC.	Ranunculaceae	Aerial part	N. E.
Crataegus pinnatifida BUNGE	Rosaceae	Stem	36.2±2.6
Cryptotaenia japonica HASSK.	Umbellifeae	Whole plant	N. E.
Cuscuta chinensis LAM.	Convolvulaceae	Fruit, Stem	23.9±0.5
Dictamnus dasycarpus TURCZ.	Rutaceae	Root	N. E.
Eriobotrya japonica LINDL.	Rosaceae	Flower	N. E.
Euonymus alatus SIEB.	Celastraceae	Stem	N. E.
Helianthus tuberosus L.	Compositae	Aerial part	14.1±8.4
Isodon excisus KUDO	Labiatae	Aerial part	6.2±5.7
Isodon excisus KUDO	Labiatae	Root	16.5±0.6
Juglans mandshurica MAX.	Juglandaceae	Fruit	10.0±4.7
Leonurus sibiricus L.	Labiatae	Whole plant	5.9±7.3
Lindera erythrocarpa MAKINO	Lauraceae	Leaf	50.8±1.0
Melandryum firmum ROHRB.	Caryophyllaceae	Whole plant	1.4±6.0
Ranunculus chinensis BUNGE	Ranunculaceae	Root	N. E.
Serratula coronata var. *insularis* KITAMURA	Compositae	Root	N. E.
Siegesbeckia pubescens MAKINO	Compositae	Whole plant	46.6±8.8
Sinomenium acutum REHDER et WILS.	Menispermaceae	Whole plant	N. E.
Sorbus commixta HEDL.	Rosaceae	Leaf	10.4±1.3
Stewartia koreana NAKAI	Theaceae	Stem	27.2±1.1
Tilia amurensis RUPR.	Tiliaceae	Leaf	32.2±2.0
Typha angustata BORT et CHAUB	Typhaceae	Aerial part	N. E.
Veratrum maackii var. *japonica* T. SHIMIZU	Liliaceae	Whole plant	N. E.
Viscum album var. *coloratum* OHWI	Loranthaceae	Root	N. E.

[1)]The concentration of the extracts tested was 100 μg/ml and results are the mean±SE (n=3).
[2)]no effect.

ical constituents of *Berchemia* species, berchemolide, (-)-berchemol and many phenolic compounds, such as (-)-catechin, vanillic acid, tachioside and syringic acid β-glucopyranosyl ester have been isolated from the stem of this species (Sakurai *et al.*, 1992). More detailed research is now in progress to find for inhibitory compounds on HIV-1 protease.

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