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Review

Inhibitory effects of Korean plant resources on human immunodeficiency virus type 1 protease activity

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INTRODUCTION

Acquired immunodeficiency syndrom (AIDS), the pandemic immunosuppressive disease of the second half of the 20th century, is still a threatening disease world-wide. UNAIDS and WHO estimated that the number of people living with Human immunodeficiency virus (HIV) at the end of the year 2001 standed at 40 million. In sub-Saraha Africa, approximately 3.4 million new infections occurred in 2001, bringing to 28.1 million the total number of people living with HIV/AIDS in this region. The etiological agent of AIDS has been identified as a retrovirus of the Lentiviridae family (Barre-sonousso *et al.*, 1983; Gallo *et al.*, 1984).

Originally referred to as HTLV-III or LAV, this enveloped, single-stranded RNA virus is now designated HIV, to eliminate confusion caused by two names for the same entity (Coffin *et al.*, 1986; Gallo and Montagnier, 1988). The development of antiretroviral therapy against AIDS has been an intense research effort since the discovery of the causative agent.

A large array of drugs and biologic substances can inhibit HIV replication *in vitro*. Zidovudine has been reported to decrease both mortality and the frequency and severity of opportunistic infections in a select group of patients infected with HIV (Fischl *et al.*, 1987). However, the administration of zidovudine has also been associated with substantial toxicity, primarily hematologic in nature (Richman *et al.*, 1987; Schmitt *et al.*, 1988; Larder *et al.*, 1989). Therefore, the development of specific and potent anti-AIDS drugs to restrain infection by HIV remains an urgent need.

Numerous plant-derived substances have been evaluated for their inhibitory effects on HIV replication *in vitro* (Sun *et al.*, 1996; Zhao *et al.*, 1997; EI-Meselhy *et al.*, 2000).

And medicinal plants from Indonesia (Kusumoto *et al*, 1992), Sri Lanka (Kusumoto *et al*, 1995), China (Ma *et al.*, 1998), Inner Mongolia in China (Ma *et al.*, 2000; 2001), Panama (Lim *et al.*, 1997) and Sudan (Hussein *et al.*, 1999) have been tested for their inhibitory activities on HIV-1 protease by Hattori group. This paper reviews the inhibitory effects of Korean plants and their compounds against HIV-1 protease.

HIV-1 PROTEASE

HIV possesses some specific enzymes on viral replication. Reverse transcriptase (RT) transcribes the viral RNA into a double-strand DNA. Then, this DNA is integrated into the host chromosome and the viral components are synthesized and assembled into new virions. The maturation of the virus takes place by viral protease. The mature viruses bud from the cells and continuously infect other T-cell. The blocking of any of these steps in the viral life cycle is expected to stop the viral replication.

Recently, clarification on the strcture and function of protease has shown another target in HIV (Kohl *et al.*, 1988; Meek *et al.*, 1990; Mitsuya *et al.*, 1990). The HIV-1 protease of molecular weight 11,000 daltons is an aspartic protease containing an Asp-Thr-Gly resudue as its active site. HIV-1 viral genome contains three large open reading frames, designated gag, pol and env, that code for proteins ultimately destined for incorporation into the

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mature virus.

The gag gene is translated as a 55 KDa fusion protein, p55gag, from which the core structural proteins of the virion, p17, p24, p7 and p6, are derived (Leis *et al.*, 1988). The pol gene contains the transcript for viral enzymes and is translated as a high molecular weight gap-pol polyprotein resulting from a ribosomal frameshift within gag (Jacks *et al.*, 1988). The gag-pol precursor, p160gap-pol, is processed to produce mature gap proteins, reverse transcriptase, intergrase and protease itself (Farmerie *et al.*, 1987). Finally, the env gene endodes the surface glycoproteins of the virion, gp41 and gp120, which are responsible for binding to the target cell CD4 receptor. Mutational inactivation of HIV-1 protease (Kohl *et al.*, 1988; Peng *et al.*, 1989) results in immature, non-infectious virions, indicating that exogenous inhibition of the protease may represent an attractive approach to anti-AIDS therapy.

IINHIBITORY EFFECTS OF KOREAN PLANTS ON HIV-1 PROTEASE

The inhibitory effects of Korean plants against HIV-1 protease activity was first reported by Park *et al.* (1996; 1997; 1998). Among the thirty-seven methanol extracts of Korean plants (Min *et al.*, 1998), the root of *Acanthopanax koreanum* (No. 2), the whole plant of *Aruncus dioicus* var. *kamtschaticus* (No. 7), the bark of *Berchemia berchemiaefolia* (No. 9), the stem of *Crataegus pinnatifida* (No. 14), the leaves of *Lindera erythrocarpa* (No. 24), the whole plant of *Siegesbeckia pubescens* (No. 41) and the leaf of *Tilia amurensis* (No. 50) were found inhibitory activities (32-56%) of the recombinant enzyme at a concentration of 100 μg/ml (Table 1). Yu *et al.* (1997; 1998) found the methanol extract of the leaves of *Syringa dilatata* (No. 48) inhibited HIV-1 protease activity by 50.5% at a concentration of 100 μg/mL. Significant inhibitory effects were observed in the stem bark of *Acanthopanax koreanum* (No. 1), the stem of *Berchemia berchemiaefolia* (No. 9) and the roots of *Rhodiola rosea* (No. 33) with inhibition of 52.3%, 58.6% and 70.4%, respectively (Min *et al.*, 1999a). Park *et al* (2000a; 2000b) reported that the water extract of the aerial part of *Orostachys japonicus* (No. 28) showed a inhibitory effect by 48.2%, and the water extract of the leaves and stem of *Rosa davurica* (No. 34-37) showed 48-50% inhibition at a concentration of 100 μg/mL. Min *et al.* (2001) measured recombinant protease assay. From the screening of 49 plants, *Atractylodes japonica* (root, No. 8) and *Clematis heracleifolia* (whole plant, No. 11) were found to inhibit the activities (40%) at a concentration of 100 μg/mL. Hur *et al.* (2002) tested 99 extracts of Korean plants against HIV-1 protease. At a concentration of 100 μg/ml, the most potent inhibitory effect was observed in the water extract of the stem of *Viburnum awabuki* (No. 53) with 91.7% inhibition. Also the water extracts of the leaves of *Distylium racemosum* (No. 17) and *Viburnum awabuki* (No. 52) showed strong inhibitions by 70.3 and 69.3%, respectively. Three extracts showed appreciable inhibitory activity (50%) against HIV-1 protease; the water extract of the stem of *Distylium racemosum* (No. 19), and the methanol extracts of the root of *Physalis alkekengi* var. *francheti* (No. 30) and the stem of *Platycarya strobilacea* (No. 31).

The inhibitory effect of the edible plants on HIV-1 protease was investigated by Park *et al.* (2000c). From 15 edible plants, the extract of the leaves of *Cedrela sinensis* (No. 7) showed a moderate inhibitory effect by 41.7% on HIV-1 protease (Table 2). The leaves of *C. sinensis* have been used for food at south area in Korea and oriental medicine for treating enteritis, dysentery and itch.

HIV-1 PROTEASE INHIBITORY SUBSTANCES FROM KOREAN PLANTS

Cammelliatannin H (Fig. 1), a dimeric hydrolyzable tannin from the pericarp of *Camellia japonica*, showed a significant inhibitory effect, giving 50% inhibitory concentration (IC_{50}) value of 0.9 μM (Park *et al.*, 2002). Kakiuchi *et al* (1985) reported that hydrolyzable tannins inhibited the polymerization catalyzed by the reverse transcriptase from avian myeroblastosis virus, and moreover dimeric ellagitannins, a group of hydrolyzable tannins, were more effective inhibitors of the reaction than monomeric ellagitannin.

Uvaol (Fig. 1) and ursolic acid isolated from *Crataegus pinatifida* in Korea showed a potent inhibitory activity with IC_{50} values of 5.5 and 8.0 ìM, respectively (Min *et al.*, 1999b). In naturally occuring ursane-type triterpenoids, the compound

Table 1. Inhibitory effects of Korean medicinal plants on HIV-1 protease

No	Scientific name	Family	Part used	Extract	Inhibition (%)
1	*Acanthopanax koreanum* NAKAI	Araliaceae	Stem bark	MeOH	52.3±4.3
2	*Acanthopanax koreanum* NAKAI	Araliaceae	Root	MeOH	38.7±6.5
3	*Acontum uchiyamai* NAKAI	Ranunculacea	Root	MeOH	35.5±4.0
4	*Agrimonia pilosa* LEDEB.	Rosaceae	Whole plant	MeOH	35.0±4.0
5	*Alisma plantago-aquatica* var. *orientale* SAMUELS.	Alismataceae	Aerial part	MeOH	48.5±1.8
6	*Artemisia princeps* var. *orientalis* HABA	Compositae	Leaves, Stem	MeOH	40.0±6.4
7	*Aruncus dioicus* var. *kamtschaticus* HABA	Rosaceae	Whole plant	MeOH	32.1±8.6
8	*Atractylodes japonica* KOIDZ.	Compositae	Root	MeOH	40.3±2.0
9	*Berchemia berchemiaefolia* KOIDZ.	Rhamnaceae	Stem	MeOH	58.6±2.5
10	*Celastrus orbiculatus* THUNB.	Celastraceae	Root	MeOH	49.1±4.8
11	*Clematis heracleifolia* DC.	Ranunculaceae	Whole plant	MeOH	45.3±2.7
12	*Corchoropsis tomentosa* L.	Sterculiaceae	Aerial part	MeOH	40.8±5.0
13	*Cornus walteri* WAGNER.	Cornaceae	Aerial part	MeOH	38.6±2.6
14	*Crataegus pinnatifida* BUNGE	Rosaceae	Stem	MeOH	36.2±2.6
15	*Cryptotaenia japonica* HASSK.	Umbelliferae	Aerial part	MeOH	37.6±4.8
16	*Cuscuta japonica* CHOIS.	Convolvlaceae	Aerial part	MeOH	47.2±7.7
17	*Distylium racemosum* S. et Z.	Hamamelidaceae	leaves	H_2O	70.3±7.8
18	*Distylium racemosum* S. et Z.	Hamamelidaceae	Stem	H_2O	64.7±5.4
19	*Erigeron annuus* (L.) PERS.	Compositae	Root	MeOH	40.6±0.1
20	*Euonymus alatus* (Thunb.) SIEB.	Celastraceae	Leaves	MeOH	35.1±4.5
21	Galla halepensis		Cocoon	MeOH	32.1±5.9
22	*Lactuca indica* var. *laciniata* (O. Kuntze) HARA	Compositae	Aerial part	MeOH	38.1±6.2
23	*Ledebouriella seseloides*(HOFFM.)OLFF.	Umbelliferae	Root	MeOH	34.6±5.2
24	*Lindera erythrocarpa* MAKINO.	Lauraceae	Leaves	MeOH	50.8±1.0
25	*Lindera obtusiloba* BL.	Lauraceae	Leaves, Stem	MeOH	35.8±8.0
26	*Lindera tomentosa* S.	Leguminosae	Leaves	MeOH	31.2±8.6
27	*Morus alba* L.	Moraceae	Leaves	MeOH	38.1±7.1
28	*Orostachys japonicus* A. BERGER.	Crassulaceae	Aerial part	H_2O	48.2±3.7
29	*Persiearia. tinctoria H.* GROSS	Polygonaceae	Whole plant	MeOH	31.1±4.9
30	*Physalis alkekengi* var. *francheti* (MASTERS) HORT.	Solanaceae	Root	MeOH	66.0±3.7
31	*Platycarya strobilacea* S. et Z.	Juglandaceae	Stem	MeOH	57.3±4.1
32	*Quercus myrsinaefolia* BL.	Fagaceae	Leaves	MeOH	35.9±12.7
33	*Rhodiola rosea* L.	Crassulaceae	Root	MeOH	70.4±2.0
34	*Rosa davurica* PALLAS	Rosaceae	Leaves	H_2O	47.8±2.6
35	*Rosa davurica* PALLAS	Rosaceae	Root	H_2O	32.0±6.7
36	*Rosa davurica* PALLAS	Rosaceae	Stem	H_2O	49.5±3.7
37	*Rosa davurica* PALLAS	Rosaceae	Root	MeOH	37.4±3.4
38	*Sageretia theezans* BRONGN.	Rhamnaceae	Leaves	MeOH	34.1±7.3
39	*Sanguisorba officinalis* L.	Rosaceae	Root	MeOH	31.3±3.4
40	*Sedum polystrchoides* HEMSL.	Crassulaceae	Whole plant	MeOH	32.1±6.6
41	*Siegesbeckia pubescens* MAKINO	Compositae	Whole plant	MeOH	46.6±8.8
42	*Smilax china* L.	Liliaceae	Stem	MeOH	32.1±4.1
43	*Sophora angustifolia* S. et Z.	Leguminosae	Root	MeOH	43.0±4.6
44	*Staphylea bumalda* DC.	Staphylaceae	Whole palnt	MeOH	40.1±4.1
45	*Stephanandra incisa* ZABEL	Rosaceae	Aerial part	MeOH	49.0±7.5
46	*Suaeda asparagoides* (MIQ.) MAKINO	Chenopodiaceae	Root	MeOH	38.1±0.4

Table 1. Inhibitory effects of Korean medicinal plants on HIV-1 protease

No	Scientific name	Family	Part used	Extract	Inhibition (%)
47	*Syneilesis palmata* (THUNB.) MAX.	Compositae	Whole plant	MeOH	38.8±5.4
48	*Syringa dilatata* S. VULGARIS	Oleaceae	Leaves	H_2O	50.5±12.9
49	*Taxodium distichum* (L.) RICH.	Taxodiaceae	Leaves	MeOH	48.7±4.0
50	*Tilia amurensis* RUPR.	Tiliaceae	Leaves	MeOH	32.2±2.0
51	*Ulmus davidiana* var. *japonica* NAKAI	Ulmaceae	Leaves, Stem	MeOH	33.6±0.2
52	*Viburnum awabuki* K. KOCH	Caprifoliaceae	Leaves	H_2O	69.3±5.4
53	*Viburnum awabuki* K. KOCH	Caprifoliaceae	Stem	H_2O	91.7±7.2
54	*Viburnum furcatum* BL.	Caprifoliaceae	Leaves	H_2O	33.8±7.8
55	*Viburnum furcatum* BL.	Caprifoliaceae	Stem	H_2O	48.4±0.7

The concentration of the extracts was 100 μg/mL. Results are the mean±SD (n=3).

Table 2. Inhibitory effects of edible plants on HIV-1 protease

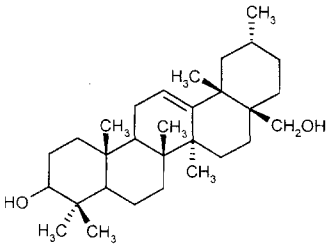
No.	Scientific name	Korean name	Family	Part used	Inhibition (%)
1	*Allium monanthum* Max.	Dal-rae	Liliaceae	whole plant	18.8±0.2
2	*Allium tuberosum* Roth.	Bu-chu	Liliaceae	aerial part	-5.9±0.3
3	*Angelica keiskei* Koidz.	Sin-seon-cho	Umbelliferae	aerial part	22.8±1.8
4	*Artemisia princeps* var. *orientalis* (Pampan.) Hara	Tsug	Compositae	aerial part	22.8±0.9
5	*Capsella bursa-pastoris* (L.) Medicus	Naeng-i	Cruciferae	whole plant	22.1±1.8
6	*Capsicum annuum* L.	Go-chu	Solanaceae	leaves	11.5±0.7
7	*Cedrela sinensis* A. Juss.	Cham-jug	Meliaceae	leaves	41.7±0.8
8	*Chrysanthemum coronarium* var. *spatiosum* Bailey	Tsug-gag	Compositae	aerial part	7.3±1.5
9	*Cirsium japonicum* var. *ussuriense* Kitamura	Eong-geong-ki	Compositae	aerial part	26.0±0.9
10	*Eriobotrya japonica* Lindl.	Bi-pa	Rosaceae	leaves	14.6±0.8
11	*Glycine max.* Merr.	Kong	Leguminosae	leaves	2.8±0.8
12	*Oenanthe javanica* (BL.) DC.	Mi-na-ri	Umbelliferae	aerial part	12.9±4.1
13	*Perilla frutescens* var. *japonica* Hara	Deul-kae	Labiatae	leaves	15.7±0.6
14	Sedum sarmentosum Bunge	Dol-na-mul	Crassulaceae	whole plant	7.0±5.0
15	*Youngia sonchifolia* Max.	Go-deul-pae-gi	Compositae	whole plant	-13.8±1.9

The results are the mean±S.E. of 3 replications.

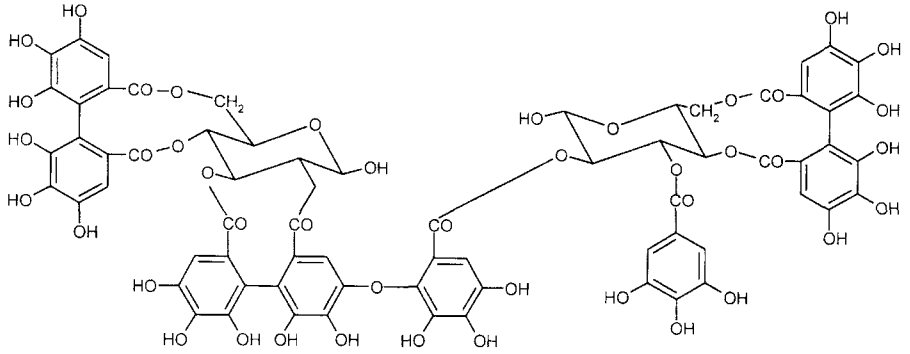
bearing a ketone group at C-3 has been reported to be a more potent inhibition of HIV-1 protease than that with a hydroxy group (Xu *et al.*, 1996; Vlietinck *et al.*, 1998). Ursolic acid was already reported to have potent HIV-protease inhibitory activity (Xu *et al.*, 1996) and some triterpenes were reported to exhibit anti-HIV activity, especially some dicarboxylic acid hemiesters of betulinic acid demonstrated extremely potent activity against the virus (Kashiwada *et al.*, 1996).

Agastraquinone and agatanol from the whole plant of *Agastache rugosa* in Korea showed significant inhibitory activity with IC_{50} of 87 μM and 360 μM, respectively against HIV-1 protease. Agastraquinone, having quinone moiety in diterpenoid compound showed also cytotoxic effect against human cancer cell lines. In natural products, several diterpenoid compounds have been described as antiviral compound. Prostatin, a phorbol ester type, showed potent anti-HIV activity without tumor-promoting effect (Gusfafson *et al.*, 1992). Tripterifordin and neotripterifordin from *Tripterygium wilfordii* inhibited HIV-1 in H9 lymphocyte cells (Chen *et al.*, 1995). 6-Hydroxytremetone from *Werneria cilliolata* showed a significant inhibition of HIV-1 replication (Piacente *et al.*, 1994).

Quercitrin isolated from the leaves of *Cedrela sinensis* in Korea, which has been used for edible plant inhibited the activity of HIV-1 protease by 19% at a concentration of 100 μM (Park *et al.*, 2000c). Flavonols, quercetin, myricetin and quercetagetin have been shown to inhibit *in vitro* RTs of certain



Uvaol



Camelliatannin H

Fig. 1.

retroviruses including Rauscher murine leukemia virus and HIV as well as cellular DNA polymerases (Ono *et al.*, 1990).

SUMMARY

Some Korean plants were found to inhibit HIV-1 protease activity. The extracts of *Acanthopanax koreanum* (stem bark), *Berchemia berchemiaefolia* (stem), *Berchemia berchemiaefolia* (bark), *Distylium racemosum* (leaves), *Distylium racemosum* (stem), *Lindera erythrocarpa* (leaves), *Physalis alkekengi* var. *francheti* (root), *Platycarya strobilacea* (stem), *Rodiola rosea* (root), *Rosa davurica* (stem), *Syringa dilatata* (leaves), *Viburnum awabuki* (stem) and *Viburnum awabuki* (leaves) showed significant inhibitory effect against HIV-1 protease. Camelliatannin H from *Camellia japonica* and uvaol from *Crataegus pinatrifida* were potent active inhibitors of HIV-1 protease with IC_{50} values of 0.9 μM and 5.5 μM, respectively. The cure and prevention of AIDS have been a global challenge since it was discovered in the ealy 1980s. However, the development of anti-HIV agent that can effectively treat or prevent this disease are still demanded.

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