Anti-HIV-1 Activity of Herbs in *Labiatae*

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The anti-HIV-1 activity of aromatic herbs in *Labiatae* was evaluated *in vitro*. Forty five extract from among 51 samples obtained from 46 herb species showed significant inhibitory effects against HIV-1 induced cytopathogenicity in MT-4 cells. In particular, the aqueous extracts of *Melissa officinalis*, a family of *Mentha*×*piperita* "grapefruit mint," *Mentha*×*piperita* var. *crispa*, *Ocimum basilicum* cv "cinnamon," *Perilla frutescens* var. *crispa* f. *viridis*, *Prunella vulgaris* subsp. *asiatica* and *Satureja montana* showed potent anti-HIV-1 activity (with an ED of 16 µg/ml). The active components in the extract samples were found to be water-soluble polar substances, not nonpolar compounds such as essential oils. In addition, these aqueous extracts inhibited giant cell formation in co-culture of Molt-4 cells with and without HIV-1 infection and showed inhibitory activity against HIV-1 reverse transcriptase.

Key words HIV-1; antiviral activity; *Labiatae*; *Melissa officinalis*; *Ocimum basilicum* cv "cinnamon"; *Perilla frutescens* var. *crispa* f. *viridis*

Several useful drugs have been recently developed for the treatment of AIDS and the prevention of its onset in carriers of its causative virus,^{1–3)} human immunodeficiency virus (HIV). It has been generally accepted that zidovudine (ZDV or AZT), didanosine (ddI), zalcitabine (ddC), belonging to the reverse transcription inhibitors and protease inhibitors suppress HIV replication. However, these drugs are very expensive and cause side effects by inhibition of hematopoietic function,⁴⁾ etc., and recently, the appearance of HIV strains resistant to these drugs is also being discussed.⁵⁾ Furthermore, there is little hope for the practical use of a vaccine at present. Therefore, it is of urgent necessity to develop new effective drugs with less toxicity.

We have already reported the *in vitro* anti-HIV-1 effects of the extracts from various crude drugs,^{6–9)} and it was suggested that crude drugs of *Labiatae* plant origin such as *Prunella* spike, *Schizonepeta* spike, *Perilla* herb, *Isodonis* herb, *Patchouli*, *Leonurus* herb and *Salvia miltiorrhiza* root showed potent anti-HIV-1 activity.⁶⁾

In this study, we examined the anti-HIV-1 activity of various herbs in *Labiatae* and demonstrated that many herbs had anti-HIV-1 activity. Additionally, we also elucidated the mechanism of the inhibitory action.

MATERIALS AND METHODS

Plant Materials and Preparation of the Extracts

Herbs (46 species in *Labiatae*) used in this study were as follows; 1. *Agastache rugosa*, 2. *Ajuga reptans*, 3. *Calamintha nepeta*, 4. *Glechoma hederacea*, 5. *Hyssopus officinalis*, 6. *Iboza riparia*, 7. *Lavandula angustifolia*, 8. *Lavandula dentata*, 9. *Lavandula stoechas*, 10. *Lavandula*×*intermedia* "Seal," 11. *Leonurus japonicus*, 12. *Melissa officinalis*, 13. *Mentha*×*piperita*, 14. *Mentha pulegium*, 15. *Mentha suaveolens*, 16. *Mentha spicata*, 17. A family of *Mentha*×*piperita* "grapefruit mint," 18. *Mentha*×*piperita* var. *citrata*, 19. *Monarda didyma*, 20. *Monarda fistulosa*, 21. *Monarda punc-*

tata, 22. *Nepeta cataria*, 23. *Ocimum basilicum*, 24. *Ocimum basilicum* cv "cinnamon," 25. *Origanum majorana*, 26. *Origanum vulgare*, 27. *Orthosiphon aristatus*, 28. *Perilla frutescens*, 29. *Perilla frutescens* var. *crispa* f. *purpurea*, 30. *Perilla frutescens* Britt. var. *crispa* f. *viridis*, 31. *Perilla frutescens* var. *japonica*, 32. *Pogostemon cablin*, 33. *Prunella vulgaris* subsp. *asiatica*, 34. *Rosmarinus officinalis*, 35. *Salvia elegans*, 36. *Salvia officinalis*, 37. *Salvia sclarea*, 38. *Satureja hortensis*, 39. *Satureja montana*, 40. *Schizonepeta tenuifolia*, 41. *Scutellaria baicalensis*, 42. *Stachys bysantina*, 43. *Stachys officinalis*, 44. *Thymus quinquecostatus*, 45. *Thymus serpyllum*, 46. *Thymus vulgaris*.

Each sample was collected at the Medical Plants Garden, Faculty of Pharmaceutical Sciences, Setsunan University, Hirakata, Osaka, Japan (1, 5, 6, 7, 11, 12, 14, 15, 16, 20, 21, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 40, 41, 44 and 46) or Nunobiki Herb Park, Kobe, Hyogo, Japan (2, 3, 8, 9, 10, 17, 18, 19, 35, 37, 39 and 42) or purchased from Forest Enterprise Ltd., Nishinomiya, Hyogo, Japan (13, 22, 38, 43, 45) or Mikuni Co., Ltd., Osaka, Japan (4), respectively. The voucher specimens were deposited at the Osaka Prefectural Institute of Public Health.

After being dried and cut, each herb (5 g) was extracted for 1 d at room temp. with acetone, ethanol, and 70% aqueous ethanol (100 ml each), successively. Evaporation of the solvents under reduced pressure gave acetone, ethanol, and 70% aqueous ethanol extracts. After the above-mentioned extraction with organic solvents, the plant residues were further extracted with hot water as follows. Each sample was gently heated with water (100 ml) until the solvent water was vaporized to about a half volume. After filtration, the filtrate was centrifuged and the supernatant was lyophilized to afford an aqueous extract. The acetone, ethanol, 70% ethanol and aqueous extracts were used for the biological tests.

Cells The HTLV-I-infected cell line MT-4 cells and human leukemic T-cell line Molt-4 were cultured at 37 °C under 5% CO₂ in RPMI-1640 medium (Flow Laboratories,

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Irvine, Scotland) supplemented with 10% fetal calf serum (FCS, Flow Laboratories, North Ryde, Australia) and 100 $\mu\text{g}/\text{ml}$ of streptomycin (Meiji Seika, Tokyo, Japan) and 100 U/ml of penicillin G (Banyu Pharmaceutical, Tokyo, Japan).

Viruses The LAV-1 and HTLV-III_B strains of HIV-1 were obtained from supernatant of cultured Molt-4 cells that had been persistently infected with LAV-1 or HTLV-III_B. KK-1 strain of HIV-1 was freshly isolated from a Japanese patient with AIDS.

Screening Test for Inhibition of HIV-1 Replication As soon as MT-4 cells are infected with HIV-1, HIV-1 rapidly grows in 4–6 d, and the MT-4 cells are eradicated.¹⁰ We assayed the inhibitory effect of each herb extract on HIV-1 replication by the following procedures:

MT-4 cells were adsorbed with HIV-1 (LAV-1 strain) at an infectious dose of 0.001 TCID₅₀/cell for 1 h, and were washed once with the culture medium mentioned above. The HIV-1 infected MT-4 cells were suspended in culture medium containing a extract sample (1000 $\mu\text{g}/\text{ml}$ at maximum concentration), which had been diluted stepwise at a rate of 1/2, at a concentration of 1.5×10^5 cells/ml. The suspension was cultured in flat bottom 96-well culture plates in quantities of 200 μl /well for 5 d. The cultured cells were microscopically examined to determine the presence or absence of a cytopathogenic effect (CPE) of HIV-1 infection on MT-4 cells and the cytotoxicity of the extract.

Test for Inhibition of HIV-1 Replication in MT-4 Cells The inhibitory effect on HIV-1 replication in MT-4 cells was evaluated by the following method: After adsorption of HIV-1 (LAV-1 strain) at an infectious dose of 0.001 TCID₅₀/cell for 1 h at 37°C, the cells were washed once with the above culture medium. HIV-infected and uninfected MT-4 cells were suspended in the culture medium containing a herb extract (1000 $\mu\text{g}/\text{ml}$ at maximum concentration), which had been diluted stepwise at a rate of 1/3, at a concentration of 1.5×10^5 cells/ml in flat bottom 48-well culture plates in quantities of 600 μl /well for 5 d. After cultivation, the viable cells were stained with trypan blue and their number was calculated.

Suppression of HIV-1 Induced Giant Cell Formation Of the extracts with strong inhibitory effect on HIV-1 replication in MT-4 cells, the presence or absence of giant cell formation in Molt-4 cells was examined. Equal volume of cell suspensions (5×10^5 cells/ml) of Molt-4 cells persistently infected with and without with HIV-1 (HTLV-III_B strain) were mixed and the mixture was added to the extract samples of various concentrations in flat bottom 48-well culture plates in quantities of 600 μl /well and cultivated for 22 h. The culture was microscopically examined to determine the presence or absence of giant cells.¹¹

Extracellular Inhibition of the Activity of HIV-1 Reverse Transcriptase The inhibitory effect on reverse transcriptase (RT) activity of samples was evaluated using the highly sensitive non-radioisotope assay for RT activity developed by Nakano *et al.*¹² A serially diluted extract sample (50 μl) was added to 50 μl of RT standard solution, and to 50 μl of the mixture, RT substrate solution was added. The resulting solution was mixed by platemixer, and incubated at 37°C for 1 h. The reaction was stopped by adding 10 μl of 5 M NaCl solution (stopping solution) and mixed at room

temp. for 5 min. The mixture was washed five times with washing solution containing 0.1 M NaCl, and 0.05 M MgCl₂ in 50 mM Tris-HCl, pH 7.5, and 100 μl of streptavidin-alkaline phosphatase solution was placed in each well and the plate was incubated at 37°C for 1 h. The plate was washed five times with the above washing solution, then 150 μl of the substrate solution alkaline phosphatase containing 1 mg of *p*-nitrophenyl phosphate per ml and 1 mM MgCl₂ in 1 M diethanolamine-HCl buffer, pH 9.5 was added, and the solution was mixed by platemixer, and incubated at 37°C for 1 h. The reaction was stopped by adding 50 μl of 1 N NaCl, and the absorbance at 405 nm was measured.

RESULTS

Anti-HIV-1 Activity of Herbs in Labiatae The activity of the herb extracts for inhibition of HIV-1 replication in MT-4 cells was examined. Acetone and ethanol extracts, which must contain nonpolar aromatic substances such as essential oil, did not show anti-HIV-1 activity at all. Of the 51 extract samples obtained from 46 herbs, 43 samples of aqueous extracts and 11 samples of 70% aqueous ethanol extracts gave anti-HIV-1 activity. These results suggested that the inhibitory effect on HIV-1 replication in these herbs is not due to nonpolar aromatic substances characteristic to herbs and must arise from some water-soluble polar compounds in the aqueous and the 70% aqueous ethanol extracts from the herbs. Anti-HIV-1 activities and cytotoxicity of the aqueous and the 70% aqueous ethanol extracts which inhibited the HIV-1 replication are shown in Table 1.

The aqueous extracts of **12**, **17**, **18**, **24**, **30**, **33** and **39** showed strong anti-HIV-1 activity with an effective dose (ED) of 16 $\mu\text{g}/\text{ml}$. Extracts of root, stem and leaf, and the spike of **33** showed the activity, but the spike of a crude drug, "Prunella Spike (Kagosoh in Japan)," gave the strongest activity. Many other samples showed comparatively strong anti-HIV-1 activities with an ED of 31 $\mu\text{g}/\text{ml}$.

The above-mentioned seven extracts which showed potent anti-HIV-1 activity were further investigated in detail. The 50% effective dose (ED₅₀) and 50% cytotoxic dose (CD₅₀) values of each sample were observed in 5-d culture (Table 2). The aqueous extracts of **12**, **17**, **18**, **24**, **30**, **33** and **39** showed ED₅₀ values between 3.8 and 17.5 $\mu\text{g}/\text{ml}$ and that of **12** appeared to be the most potent (3.8 $\mu\text{g}/\text{ml}$). These values are comparable to the strong anti-HIV-1 activity of dextran sulfate 8000 (3.9 $\mu\text{g}/\text{ml}$). The selective indexes (SI: the ratio of CD₅₀ to ED₅₀) of the extracts of **12**, **30** and **39** were more than 10 and the extract of **30** gave a high SI value (50).

Seventeen aqueous extracts showed potent anti-HIV-1 activity (ED) at lower concentration than 31 $\mu\text{g}/\text{ml}$ (Table 1). Therefore, the inhibitory effects of these 17 extracts on freshly isolated HIV-1 (KK-1 strain) replication which we obtained from an AIDS patients were examined (Table 3). The aqueous extract of **12** showed the most potent inhibitory effect (an ED of 62 $\mu\text{g}/\text{ml}$) on KK-1 strain. **24** and 3 perilla species (**29**, **30** and **31**) also gave inhibitory activity.

The Mechanism of Action of Anti-HIV-1 Activity The inhibitory effect of cell to cell infection was examined by use of a HIV-1 induced giant cell formation system. As shown in Table 4, all of the extracts showed inhibition of giant cell formation below the low concentration of 250 $\mu\text{g}/\text{ml}$. Therefore,

Table 1. Inhibitory Effects of Herb Extracts on HIV-1 Replication

Botanical name	Part	Origin ^{a)}	Extract ^{b)}	ED ^{c)}	CD ^{d)}
1. <i>Agastache rugosa</i>	Leaf	S	E	62	125
2. <i>Ajuga reptans</i>	Aerial	N	W	500	1000
3. <i>Calamintha nepeta</i>	Leaf	N	W	125	500
4. <i>Glechoma hederacea</i>	Leaf	M	W	31	250
6. <i>Iboza riparia</i>	Aerial	S	W	31	250
7. <i>Lavandula angustifolia</i>	Leaf	S	E	500	1000
			W	62	250
8. <i>Lavandula dentata</i>	Leaf	N	W	31	62
	Flower	N	W	62	250
9. <i>Lavandula stoechas</i>	Leaf	N	E	62	125
			W	62	500
	Flower	N	W	62	250
11. <i>Leonurus japonicus</i>	Aerial	S	W	62	500
12. <i>Melissa officinalis</i>	Leaf	S	W	16	62
13. <i>Mentha</i> × <i>piperita</i>	Leaf	F	E	125	250
			W	62	250
14. <i>Mentha pulegium</i>	Leaf	S	E	125	500
			W	62	125
15. <i>Mentha suaveolens</i>	Leaf	S	E	250	1000
			W	62	250
16. <i>Mentha spicata</i>	Leaf	S	W	31	250
17. A family of <i>Mentha</i> × <i>piperita</i> "grapefruit mint"	Leaf	N	W	16	125
18. <i>Mentha</i> × <i>piperita</i> var. <i>citrata</i>	Leaf	N	W	16	250
20. <i>Monarda fistulosa</i>	Aerial	S	W	62	250
21. <i>Monarda punctata</i>	Leaf	S	W	125	500
22. <i>Nepeta cataria</i>	Aerial	F	W	62	62
23. <i>Ocimum basilicum</i>	Leaf	S	E	125	>1000
			W	31	125
24. <i>Ocimum basilicum</i> cv "cinnamon"	Aerial	S	E	1000	>1000
			W	16	250
25. <i>Origanum majorana</i>	Aerial	S	W	31	250
26. <i>Origanum vulgare</i>	Leaf	S	W	31	250
27. <i>Orthosiphon aristatus</i>	Leaf	S	E	125	500
			W	31	500
28. <i>Perilla frutescens</i>	Leaf	S	W	31	250
29. <i>Perilla frutescens</i> var. <i>crispa</i> f. <i>purpurea</i>	Leaf	S	W	31	500
30. <i>Perilla frutescens</i> Britt. var. <i>crispa</i> f. <i>viridis</i>	Leaf	S	W	16	250
31. <i>Perilla frutescens</i> var. <i>japonica</i>	Leaf	S	W	31	500
32. <i>Pogostemon cablin</i>	Aerial	S	W	500	1000
33. <i>Prunella vulgaris</i> subsp. <i>asiatica</i>	Root	S	W	31	250
	Stem	S	W	31	250
	Spike	S	W	16	500
34. <i>Rosmarinus officinalis</i>	Aerial	S	E	250	500
			W	62	250
35. <i>Salvia elegans</i>	Leaf	N	E	31	62
			W	31	62
36. <i>Salvia officinalis</i>	Aerial	S	W	62	250
37. <i>Salvia sclarea</i>	Leaf	N	W	250	500
	Flower	N	W	125	500
38. <i>Satureja hortensis</i>	Aerial	F	W	62	62
39. <i>Satureja montana</i>	Leaf	N	W	16	62
40. <i>Schizonepeta tenuifolia</i>	Aerial	S	W	62	125
44. <i>Thymus quinquecostatus</i>	Aerial	S	W	31	125
45. <i>Thymus serpyllum</i>	Aerial	F	W	31	125
46. <i>Thymus vulgaris</i>	Aerial	S	W	62	250

a) Collected place or purchased company: F; Foresta enterprise Ltd., Nishinomiya, Hyogo, Japan, S; Medicinal plants garden, Setsunan University, N; Nunobiki herb park, Kobe, Hyogo, Japan, M; Mikuni Co. Ltd., Osaka, Japan. b) E, 70% ethanol extract; W, aqueous extract. c) Effective dose indicates inhibitory activity of HIV-1 induced cytopathogenicity in MT-4 cells ($\mu\text{g/ml}$). d) Cytotoxic dose indicates the reduction of the viability of MT-4 cells ($\mu\text{g/ml}$).

these extracts were considered to block the binding and/or fusion of virus to cells.

Furthermore, the effects of the extracts on RT activity were evaluated. The ED_{50} of the extracts was determined and shown in Table 5. The aqueous extract of **8**, **12**, **17**, **18**, **28**, **29**, **35**, **39** and **45** gave potent RT inhibitory activity, the ED_{50} of which were less than $10 \mu\text{g/ml}$. In particular, **17** and **18** strongly inhibited the enzyme activity (ED_{50} : 0.97 and

$0.99 \mu\text{g/ml}$, respectively). On the contrary, all extracts of **6**, **7**, **16**, **25** and **44** only showed weak activity in RT inhibition ($\text{ED}_{50} > 100 \mu\text{g/ml}$).

DISCUSSION

We have investigated anti-HIV-1 activity (*in vitro*) of many crude drugs and natural products. In the present study, by the

Table 2. Anti-HIV-1 Activity of Some Herb Extracts on HIV-1 in MT-4 Cell Cultures

Sample	ED ₅₀ ^{a)}	CD ₅₀ ^{b)}	SI ^{c)}
12. <i>Melissa officinalis</i>	3.8	83	21
17. A family of <i>Mentha</i> × <i>piperita</i> "grapefruit mint"	9.5	93	9.8
18. <i>Mentha</i> × <i>piperita</i> var. <i>citrata</i>	13.5	100	7.4
24. <i>Ocimum basilicum</i> cv "cinnamon"	17.5	97	5.5
30. <i>Perilla frutescens</i> var. <i>crispa</i> f. <i>viridis</i>	5.4	270	50
33. <i>Prunella vulgaris</i> subsp. <i>asiatica</i> (spike)	8.7	46	5.3
39. <i>Satureja montana</i>	9.2	94	10
Dextran sulfate (8000, SIGMA)	3.9	>1000	>256

a) Fifty percent effective dose indicates the inhibitory effect of HIV-1-induced cytopathogenicity in MT-4 cells (μg/ml). b) Fifty percent cytotoxic dose indicates the reduction of the viability of mock-infected MT-4 cells (μg/ml). c) Selective index (CD₅₀/ED₅₀).

Table 3. Suppressive Effect of Herb Extracts on the Replication of Freshly Isolated HIV-1 Strain (KK-1)

Sample	ED ^{a)}	CD ^{b)}
6. <i>Iboza riparia</i>	—	250
7. <i>Lavandula angustifolia</i>	250	500
8. <i>Lavandula dentata</i> (leaf)	—	125
12. <i>Melissa officinalis</i>	62	125
16. <i>Mentha spicata</i>	—	250
17. A family of <i>Mentha</i> × <i>piperita</i> "grapefruit mint"	—	125
18. <i>Mentha</i> × <i>piperita</i> var. <i>citrata</i>	125	250
23. <i>Ocimum basilicum</i>	250	500
24. <i>Ocimum basilicum</i> cv "cinnamon"	125	500
25. <i>Origanum majorana</i>	—	125
26. <i>Origanum vulgare</i>	—	250
27. <i>Orthosiphon aristatus</i>	—	125
28. <i>Perilla frutescens</i>	—	125
29. <i>Perilla frutescens</i> var. <i>crispa</i> f. <i>purpurea</i>	125	250
30. <i>Perilla frutescens</i> var. <i>crispa</i> f. <i>viridis</i>	125	250
31. <i>Perilla frutescens</i> var. <i>japonica</i>	125	250
33. <i>Prunella vulgaris</i> subsp. <i>asiatica</i> (root)	—	125
(stem)	—	125
(spike)	—	125
35. <i>Salvia elegans</i>	250	500
39. <i>Satureja montana</i>	—	125
44. <i>Thymus quinquecostatus</i>	—	250
45. <i>Thymus serpyllum</i>	250	500

a) Effective dose indicates inhibitory effect of newly isolated HIV-1 strain induced cytopathogenicity in MT-4 cells. b) Cytotoxic dose indicates the reduction of the viability of MT-4 cells (μg/ml).

screening test of inhibition of HIV-1 replication in MT-4 cells *in vitro*, we found that the aqueous extracts of many herbs belonging to *Labiatae* inhibited HIV-1 replication. Seven extracts of 46 herb species showed remarkable anti-HIV-1 activity (ED₅₀: 16 μg/ml) (Table 1). Especially, the extract of 12 gave the strongest anti-HIV-1 activity (ED₅₀: 3.8 μg/ml and SI: 22). The extract of 30 showed the most favorable SI value of 50, suggesting low cell toxicity (Table 2).

There is strong mutual affinity between gp120 which is an envelope protein of HIV-1 and the CD4 molecule on the surface of T4 cells. CD4-positive cells infected with HIV-1 are fused with many uninfected CD4-positive cells. And the resultant giant cells lose normal cell function and finally die. Destruction of CD4-positive cells affects the immunological defense mechanism inducing immunodeficiency in a patient infected with HIV-1. A drug that can inhibit this stage is highly likely to be effective.¹³⁾ In this experiment, the herb extracts inhibited the giant cell formation in co-culture of Molt-4 cells infected with HIV-1 and uninfected Molt-4 cells at a concentration of 32 to 250 μg/ml (Table 4). This activity

Table 4. Suppressive Effect of Herb Extracts on Giant Cell Formation

Sample	ED ^{a)}	CD ^{b)}
6. <i>Iboza riparia</i>	250	500
7. <i>Lavandula angustifolia</i>	62	>500
8. <i>Lavandula dentata</i> (leaf)	62	500
12. <i>Melissa officinalis</i>	62	500
16. <i>Mentha spicata</i>	125	>500
17. A family of <i>Mentha</i> × <i>piperita</i> "grapefruit mint"	32	500
18. <i>Mentha</i> × <i>piperita</i> var. <i>citrata</i>	62	500
23. <i>Ocimum basilicum</i>	125	>500
24. <i>Ocimum basilicum</i> cv "cinnamon"	62	>500
25. <i>Origanum majorana</i>	125	>500
26. <i>Origanum vulgare</i>	62	>500
27. <i>Orthosiphon aristatus</i>	62	250
28. <i>Perilla frutescens</i>	62	500
29. <i>Perilla frutescens</i> var. <i>crispa</i> f. <i>purpurea</i>	62	500
30. <i>Perilla frutescens</i> var. <i>crispa</i> f. <i>viridis</i>	62	>500
31. <i>Perilla frutescens</i> var. <i>japonica</i>	62	>500
33. <i>Prunella vulgaris</i> subsp. <i>asiatica</i> (root)	62	500
(stem)	62	500
(spike)	125	500
35. <i>Salvia elegans</i>	125	>500
39. <i>Satureja montana</i>	62	>500
44. <i>Thymus quinquecostatus</i>	125	>500
45. <i>Thymus serpyllum</i>	125	500

a) Effective dose indicates inhibitory effect of giant cell formation in co-cultures of MOLT-4 cells with MOLT-4/HTLV-III_B cells. b) Cytotoxic dose indicates the reduction of the viability of MT-4 cells (μg/ml).

was correlated with the anti-HIV-1 activity in MT-4. These findings indicate that the inhibition mechanism by the herbs is due to inhibition of the binding and/or fusion of the virus to cells.

The KK-1 strain of HIV-1 was isolated from an AIDS patient, and this strain is closer to the natural HIV-1 character isolated from AIDS patients than those of laboratory HIV-1 strains such as LAV-1 and HTLV-III_B which have been grown in an established T-cell line (Table 3). We confirmed the anti-HIV-1 effect of the herbs by the use of the KK-1 strain. Our findings suggest that these herbs also inhibit HIV-1 replication in the body of HIV carriers.

The herbs belonging to *Labiatae* were found to inhibit the extracellular RT activity of HIV-1 (Table 5). However, the ED₅₀ values of 6, 7, 16 and 44 were about 100 μg/ml, respectively. In the experiment using of MT-4 cells, those herbs completely inhibited HIV-1 replication at a concentration of 16 μg/ml (Table 1), but did not inhibit RT activity at a similar concentration. These findings suggest that the anti-HIV-1 effect of herbs does not play an important role in RT inhibition. However, the extracts must exhibit inhibitory activity on an-

Table 5. Effect of Herb Extracts on HIV-1 Reverse Transcriptase Activity

Sample	ED ₅₀ (μg/ml) ^{a)}
6. <i>Iboza riparia</i>	130
7. <i>Lavandula angustifolia</i>	123
8. <i>Lavandula dentata</i> (leaf)	7.6
12. <i>Melissa officinalis</i>	1.6
16. <i>Mentha spicata</i>	117
17. A family of <i>Mentha</i> × <i>piperita</i> "grapefruit mint"	0.97
18. <i>Mentha</i> × <i>piperita</i> var. <i>citrata</i>	0.99
23. <i>Ocimum basilicum</i>	76
24. <i>Ocimum basilicum</i> cv "cinnamon"	15
25. <i>Origanum majorana</i>	140
26. <i>Origanum vulgare</i>	79
27. <i>Orthosiphon aristatus</i>	24
28. <i>Perilla frutescens</i>	7.1
29. <i>Perilla frutescens</i> var. <i>crispa</i> f. <i>purpurea</i>	8.2
30. <i>Perilla frutescens</i> var. <i>crispa</i> f. <i>viridis</i>	27
31. <i>Perilla frutescens</i> var. <i>japonica</i>	50
33. <i>Prunella vulgaris</i> subsp. <i>asiatica</i> (root)	45
	(stem) 60
	(spike) 92
35. <i>Salvia elegans</i>	3.2
39. <i>Satureja montana</i>	5.5
44. <i>Thymus quinquecostatus</i>	128
45. <i>Thymus serpyllum</i>	7.6

a) Activity of HIV-1 RT was determined by Nakano *et al.*'s method.¹²⁾

other step of replication of HIV-1 strains. On the other hand, the ED₅₀ of RT activity of 17 and 18 were about 1 μg/ml, but in the experiment using of MT-4 cells, these extracts (17 and 18) inhibited the HIV-1 replication (ED₅₀: 9.5 and 13.5 μg/ml, respectively). Accordingly, these RT inhibitory effects were not considered to have a direct relation to HIV-1 replication.

Caffeic and quinic acids, which are widespread in *Labiatae* plants, are not soluble in water and had no activity,¹⁴⁾ suggesting that neither of them is the active principle. But their relatives such as their polymers, their metallic salts, and/or their polar derivatives may correspond to the active principles. The amounts of metals such as Ca, Mg and Fe in these *Labiatae* herbs were higher than plants in other families. Especially, 12, 24 and 33 (spike) contained 1000—2500 ppm of Fe and 7000—10000 ppm of Mg, suggesting that these metals may influence the anti-HIV activity.¹⁴⁾ Yao *et al.* found that the extracts of a crude drug, "Prunella

Spike" inhibited the HIV-1 replication and *in vitro* gp120 combined with CD4,¹⁵⁾ but the anti-HIV-1 active substance has not yet been determined. The elucidation of the chemical structure of anti-HIV-1 active principles in these herbs in *Labiatae* is necessary and is now under progress.

Since the concentrations effective against HIV-1 in the extracts of herbs as demonstrated in MT-4 cells (Table 1) were at the remarkably low concentration of 16—32 μg/ml, these extracts were considered to be clinically applicable if the active principles in the extracts could be administered in an effective form in the blood *in vivo*. Furthermore, the herbs were effective for the freshly isolated strain KK-1 and were thought to be less toxic because they have been used for a long time. The herbs are likely to be effective for the chemotherapy for patients with HIV-1 who need to take the drug for many years and for the prevention of onset of AIDS in asymptomatic HIV carriers.

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