Screening of Egyptian folk medicinal plant extracts for anti-human immunodeficiency virus type-1 (HIV-1) activity

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Abstract

As a result of screening the extracts of 41 Egyptian folk medicines, six plants showed inhibitory activity against HIV-1 : methanol extracts of *Abrus precatorius* L., *Artemisia absinthium* L., *Croton tiglium* L. and *Datura stramonium* L., and water extracts of *Bassia muricata* (L.) MURR., *Centaurea scoparia* L., *Croton tiglium* L. and *Datura stramonium* L.. Their respective anti-HIV-1 activity (IC_{50}) for MT-4 cells were 2.0, 9.8, 0.025, 4.1, 135, 46.2, 2.0 and 90.0 (μg/ml). *Croton tiglium* L. also suppressed giant cell formation in co-cultures of MOLT-4 cells with MOLT-4/HTLV-IIIB cells. Moreover, *Abrus precatorius* L., *Artemisia absinthium* L., *Croton tiglium* L. had direct effects and decreased the infectivity of HIV-1. However, none of them showed any inhibitory effect on the reverse transcriptase and protease activity of HIV-1.

Key words Egyptian folk medicinal plant, anti-HIV-1 activity.

Abbreviations CC, cytotoxic concentration ; CC_{50}, 50% cell toxicity concentration ; CPE, cytopathic effect ; HIV-1, human immunodeficiency virus-type 1 ; IC, inhibitory concentration ; IC_{50}, 50 % inhibitory concentration ; SI, selectivity index ; $TCID_{50}$, 50 %-tissue culture infective dose.

Introduction

In the development of the drugs against acquired immunodeficiency syndrome (AIDS), several stages in the replication cycle of human immunodeficiency virus (HIV) have been considered targets for chemotherapeutic intervention. Numerous compounds have been described which inhibit the replication of HIV, nevertheless, few agents have been formally licensed, in the family of nucleoside analogs, such as azidothymidine (AZT)[1] and dideoxyinosine (ddI).[2] However, long-term therapies with these reverse transcriptase (RT) inhibitors often lead to the development of resistant virus[3] and serious side effects. Since it is possible that the efficacy of each drug might be enhanced and the toxic effects might be reduced with the combined treatment of multiple drugs that have different antiretroviral mechanisms, the development of a variety of effective agents has been required.

Traditional medicines have been safely used for the treatment of various diseases. We have evaluated and reported the anti-HIV activities of several natural compounds.[4-7] In this article, we describe the inhibitory effect of Egyptian folk medicinal plant extracts.

Materials and Methods

Plant materials and preparation of the extracts : The plants were purchased at Harraz Herbal Drugstore in Cairo, Egypt. They were identified by Professor El-Sayed E. Aboutabl, Faculty of Pharmacy,

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Cairo University, Egypt. A sample specimen of each plant was deposited at the Museum of Materia Medica of Toyama Medical and Pharmaceutical University, Toyama, Japan. The powdered plant (5 g each) was refluxed separately with methanol, and water (50 ml×3) for 3 h. The extracts were concentrated and freeze-dried. To test their inhibitory effects on HIV-1 replication, the water extracts were dissolved in culture medium and the methanol extracts were dissolved in methanol before adding to the medium. The final concentration of methanol did not exceed 2 %.

Cells : The HTLV-I-carrying cell line MT-4 cells and the human leukemic T-cell line MOLT-4 cells were used. They were maintained at 37°C under 5 % CO_2 in RPMI-1640 medium (Flow Laboratories, Irvine, Scotland), supplemented with 10 % fetal calf serum (FCS, Flow Laboratories, North Ryde, Australia), 100 μg/ml of streptomycin (Meiji Seika, Tokyo, Japan) and 100 U/ml of penicillin G (Banyu Pharmaceutical, Tokyo, Japan).

Virus : The LAV-1 and HTLV-IIIB strains of HIV-1 were obtained from culture supernatant of MOLT-4 cells that had been persistently infected with LAV-1 or HTLV-IIIB.

Primary screening for anti-HIV-1 activity : MT-4 cells were infected for 1 h with HIV-1 (LAV-1) at $TCID_{50}$ of 0.001/cell. Then, the cells were washed and resuspended at 1×10^5 cells/ml in RPMI-1640 medium. A 200 μl/well of the cell suspension was cultured for five days in a 96-well culture plate containing various concentrations (12 doses, maximum 1000 μg/ml and minimum 0.49 μg/ml) of the plant extracts. Control assays were performed, without plant extract, with HIV-1-infected and -uninfected cultures. On day 5, the IC of the test sample required to completely prevent HIV-1-induced CPE was determined through an optical microscope and the cell growth was examined to give the CC that reduces the viability of MT-4 cells.

Inhibitory effect of selected plant extracts on HIV-1 replication : After the primary screening test, eight extracts (from six plants) were found to inhibit HIV-1 induced CPE and they were further investigated by more accurate method as follows. The assay was carried out in a 48-well culture plate (600 μl/well) for five days with the same method used for the primary screening. After incubation, the CPE was observed and the number of viable cells was counted by the trypan blue exclusion method. The score of viable cells in HIV-1-infected MT-4 cells exhibited the amount of plant extract required to inhibit HIV-1 replication by 50 % (IC_{50}), and in uninfected MT-4 cells culture, the dose that reduced the viability of uninfected cells by 50 % (CC_{50}) was observed. The ratio of CC_{50}/IC_{50} was calculated as the selectivity index (SI).

Suppressive effect on giant cell formation[8,9] : The eight plant extracts were also checked for any suppressive effect on giant cell formation in a co-culture of HIV-1-infected and -uninfected MOLT-4 cells. An equal number of MOLT-4 and MOLT-4/HTLV-IIIB cells were mixed (total cell number of 5×10^5 cells/ml, 600 μl/well), and cultured for 20 h in the presence of various concentrations of plant extracts. After that, the formation of a giant cell was examined with an optical microscope, and cell viability was measured with the trypan blue method. Finally, the fusion index (FI) was calculated as follows :

$$FI = 1 - \frac{\text{Cell number in test well (Molt-4+Molt-4/HTLV-IIIB)}}{\text{Cell number in control (Molt-4 cells)}}$$

With this definition, the FI can vary between 0 (no fusion) and 1 (total fusion, no viable cells remaining). The FI values obtained for each concentration of the compound can be expressed as a fraction of the control value. Thus, percentage of fusion inhibition was calculated as,

$$\% \text{ fusion inhibition} = \left(1 - \frac{FI_1}{FI_2}\right) \times 100$$

where FI_1 is the fusion index of the test sample and FI_2 is the fusion index of the control sample. Thus the 50 % inhibitory concentration (IC_{50}) was calculated.

Direct effect on HIV-1 infectivity : Plant extracts demonstrating the most effective IC_{50} (methanol extracts of **1,7** and **19** and water extracts of **19** and **20**) (Table II), were incubated with HTLV-IIIB for 1 h at 37°C. Then the suspensions were ultracentrifuged at 40,000 rpm for 1 h at 4°C (Beckman rotor SW 50). The precipitates were resuspended in medium without the test extracts. The infectious titers of HIV-1 were assayed, both before and after treatment, by microscopy for CPE in MT-4 cells.

Results

Primary screening for anti-HIV-1 activity

Forty-one Egyptian folk medicines investigated for anti-HIV-1 activity are shown in Table I. When these extracts were screened for their effects on viral replication, eight extracts from six plants were found to inhibit HIV-1 induced CPE. Each IC value was a quarter the respective CC values (Table I). Methanol extracts were made from : the seed of *Abrus precatorius* L. (**1**, IC : 3.9 μg/ml), the aerial part of *Artemisia absinthium* L. (**7**, IC : 15.6 μg/ml), the seed of *Croton tiglium* L. (**19**, IC : 0.04 μg/ml), and the seed of *Datura stramonium* L. (**20**, IC : 3.9 μg/ml). Water extracts were made from : the whole plant of *Bassia muricata*

Table I Primary screening for anti-HIV-1 activity of Egyptian folk medicinal plant extracts.

No	Botanical name (Family name)	Part used	Extract	IC (μg/ml)	CC (μg/ml)
1.	*Abrus precatorius* L. (Leguminosae)	seed	Methanol	3.9	31.2
			Water	NE	125
2.	*Aloe vera* L. (Liliaceae)	resin	Methanol	NE	250
			Water	NE	250
3.	*Ambrosia maritima* L. (Compositae)	aerial part	Methanol	NE	31.2
			Water	NE	125
4.	*Ammi majus* L. (Umbelliferae)	fruit	Methanol	NE	125
			Water	NE	1000
5.	*Anagallis arvensis* L. (Primulaceae)	whole plant	Methanol	NE	250
6.	*Artemisia herba alba* Asso. (Compositae)	aerial part	Methanol	125	250
			Water	NE	500
7.	*Artemisia absinthium* L.(Compositae)	aerial part	Methanol	15.6	62.5
			Water	NE	125
8.	*Balanites aegyptiaca* (L.) Delile (Balanitaceae)	fruit	Methanol	NE	15.6
			Water	NE	62.5
9.	*Bassia muricata* (L.) Murr. (Chenopodiaceae)	whole plant	Methanol	NE	500
			Water	500	>1000
10.	*Boswellia carterii* Birdw. (Burseraceae)	resin	Methanol	NE	31.2
			Water	NE	250
11.	*Bryonia cretica* L. (Cucurbitaceae)	resin	Methanol	NE	31.2
			Water	NE	31.2
12.	*Cassia acutifolia* Del. (Leguminosae)	leaf	Methanol	250	250
			Water	500	1000
13.	*Catharanthus roseus* G. Don (Apocynaceae)	leaf	Methanol	NE	3.9
			Water	NE	3.9
14.	*Centaurea scoparia* L. (Compositae)	aerial part	Methanol	NE	15.6
			Water	NE	62.5
		root	Methanol	NE	62.5
			Water	125	1000
15.	*Citrullus colocynthis* (L.) Schrad (Cucurbitaceae)	pericarp	Methanol	NE	1000
			Water	NE	125
		seed	Methanol	NE	500
			Water	NE	500
16.	*Cleome droserifolia* (Forssk.) Del.(Cleomaceae)	bark	Methanol	62.5	125
			Water	NE	>1000
17.	*Colchicum ritchii* R. Br.(Liliaceae)	seed	Methanol	NE	3.9
			Water	NE	7.8
18.	*Commiphora molmol* L. (Burseraceae)	resin	Methanol	NE	125
			Water	NE	1000
19.	*Croton tiglium* L. (Euphorbiaceae)	seed	Methanol	0.04	0.37
			Water	6.2	55.5
20.	*Datura stramonium* L. (Solanaceae)	seed	Methanol	3.9	15.6
			Water	125	500

21. *Digitalis purpurea* L. (Scrophulariaceae)	leaf	Methanol	NE	31.2
22. *Ferula foetida* REGEL (Umbelliferae)	resin	Methanol	62.5	62.5
		Water	NE	1000
23. *Gymnocarpos decandrum* FORSSK. (Caryophyllaceae)	whole plant	Methanol	NE	1000
24. *Hibiscus sabdariffa* L. (Malvaceae)	calyx	Methanol	NE	1000
		Water	1000	>1000
25. *Juniperus phoenicea* L. (Cupressaceae)	fruit	Methanol	NE	15.6
		Water	NE	31.2
26. *Lepidium sativum* L. (Cruciferae)	seed	Methanol	NE	500
		Water	NE	>1000
27. *Lupinus termis* FORSSK. (Leguminosae)	seed	Methanol	NE	250
		Water	NE	>1000
28. *Maerua crassifolia* FORSSK. (Capparaceae)	leaf	Methanol	NE	125
		Water	NE	250
29. *Nigella sativa* L. (Ranunculaceae)	seed	Methanol	NE	15.6
		Water	NE	250
30. *Petroselinum sativum* L. (Umbelliferae)	fruit	Methanol	125	125
		Water	NE	1000
31. *Phyllanthus emblica* L. (Euphorbiaceae)	fruit	Methanol	NE	62.5
		Water	NE	62.5
32. *Polycarpea repens* FORSSK. (Caryophyllaceae)	whole plant	Water	NE	>1000
33. *Quercus pedunculata* EHRH. (Fagaceae)	fruit	Methanol	NE	125
34. *Rumex cyprius* MURB. (Polygonaceae)	fruit	Methanol	NE	62.5
		Water	250	500
35. *Solanum nigrum* L. (Solanaceae)	fruit	Methanol	NE	31.2
		Water	NE	62.5
36. *Solenostemma argel* (DEL.) HAYNE (Asclepiadaceae)	leaf	Methanol	NE	250
		Water	NE	>1000
37. *Terminalia bellerica* ROXB. (Combretaceae)	fruit	Methanol	NE	62.5
		Water	NE	31.2
38. *Terminalia chebula* RETZ. (Combretaceae)	fruit	Methanol	NE	62.5
		Water	NE	62.5
39. *Terminalia horrida* STEUD. (Combretaceae)	fruit	Methanol	NE	62.5
		Water	NE	31.2
40. *Trigonella foenum graecum* L. (Leguminosae)	seed	Methanol	NE	125
		Water	NE	31.2
41. *Zygophyllum dumosum* BOISS (Zygophyllaceae)	seed	Methanol	31.2	62.5
		Water	1000	>1000

All the above mentioned plants were extracted with methanol and water separately except for plants number 5, 21, 23 and 33 (methanol extract), and 32 (water extract). NE : Not effective. Inhibition of HIV-1 induced CPE on MT-4 cells was not observed below their CC.

(L.) MURR. (**9**, IC : 500 μg/ml), the root of *Centaurea scoparia* L. (**14**, IC : 125 μg/ml), the seed of *Croton tiglium* L. (**19**, IC : 6.2 μg/ml), and the seed of *Datura stramonium* L. (**20**, IC : 125 μg/ml).

Inhibitory effect of selected plant extracts on HIV-1 replication

As a result of screening, eight extracts from six plants showing anti-HIV activity were investigated in more detail. The IC_{50} and CC_{50} values of each sample were observed in five day MT-4 cells culture (Table II). Methanol extracts of **1,7,19** and **20,** and water extracts of **19** showed relatively strong anti-HIV-1 activity (IC_{50}), which was 2.0, 9.8, 0.025, 4.1, 2.0 μg/ml respectively. In particular extracts of **19** revealed a high SI (Table II). The other extracts (water extracts of **9, 14,** and **20**) showed lower activity (from 46 to 135 μg/ml) than **19**..

Suppressive effect on giant cell formation

The abilities of these extracts to inhibit giant cell formation in cocultures of HIV-1-infected and -uninfected MOLT-4 cells were also evaluated (Table III). The methanol extracts of **19** were the most effective (IC_{50} : 2.9 μg/ml), followed by water extracts of **19** (IC_{50} : 25 μg/ml), water extracts of **14** (IC_{50} : 98 μg/ml)

Table II Inhibitory effect of Egyptian plant extracts on HIV-1 replication.

Material (part used, extract)	IC_{50} (μg/ml)	CC_{50} (μg/ml)	SI
1. *Abrus precatorius* L. (seed, methanol)	2.0	43.5	21.8
7. *Artemisia absinthium* L. (aerial part, methanol)	9.8	31.0	3.16
9. *Bassia muricata* (L.) MURR. (whole plant, Water)	135	>1000	>7.4
14. *Centaurea scoparia* L. (root, Water)	46.2	>1000	>21.6
19. *Croton tiglium* L. (seed, methanol)	0.025	0.86	34.4
19. *Croton tiglium* L. (seed, Water)	2.0	100	50.0
20. *Datura stramonium* L. (seed, methanol)	4.1	6.0	1.49
20. *Datura stramonium* L. (seed, methanol)	90.0	840	9.3

The data show the inhibition of CPE on MT-4 cells. Cell viability was measured by the trypan blue method. IC_{50}, 50 % inhibitory concentration ; CC_{50}, 50 % cell toxicity concentration ; SI, selectivity index (CC_{50}/IC_{50}). All data represent median values of two experiments.

Table III Suppression of HIV 1-induced giant cell formation by Egyptian plant extracts.

Material (part used, extract)	IC_{50} (μg/ml)	CC_{50} (μg/ml)
1. *Abrus precatorius* L. (seed, methanol)	NE	900
7. *Artemisia absinthium* L. (aerial part, methanol)	NE	500
9. *Bassia muricata* (L.) MURR. (whole plant, Water)	600	>1000
14. *Centaurea scoparia* L. (root, Water)	98	>1000
19. *Croton tiglium* L. (seed, methanol)	2.9	248
19. *Croton tiglium* L. (seed, Water)	25	1880
20. *Datura stramonium* L. (seed, methanol)	NE	243
20. *Datura stramonium* L. (seed, Water)	NE	1660

The data show the suppression of HIV-1 induced giant cell formation on MOLT-4 cells. Cell viability was measured by the trypan blue method. IC_{50}, 50% inhibitory concentration ; CC_{50}, 50 % cell toxicity concentration. NE, not effective. Inhibition of HIV-1-induced giant cell formation on MOLT 4 cells was not observed below CC_{50}.

Table IV Inhibitory effect of Egyptian plant extracts on the infectivity of HIV 1.

Material (extract)	Concentration (μg/ml)	$TCID_{50}$/ml
1. *Abrus precatorius* L. (methanol)	20	$10^{3.25}$
	2.0	$10^{3.75}$
7. *Artemisia absinthium* L. (methanol)	100	$10^{3.25}$
	10	$10^{4.25}$
19. *Croton tiglium* L. (methanol)	0.25	$10^{4.0}$
	0.025	$10^{3.75}$
19. *Croton tiglium* L. (Water)	0.87	$10^{4.25}$
	0.087	$10^{4.0}$
20. *Datura stramonium* L. (methanol)	8.2	$10^{4.25}$
	0.82	$10^{5.0}$
Control	0	$10^{4.75}$

$TCID_{50}$: 50 % tissue culture infective dose.

and water extracts of **9** (IC_{50} : 600 μg/ml). But the other extracts (**1,7,20**) did not show any inhibitory effect at the concentrations lower than the cytotoxic concentrations limit.

Direct effect on HIV-1 infectivity

Methanol extracts of **1,7,19** and water extracts of **19** and **20** were tested for direct effect on HIV-1 within the concentration range including respective IC_{50}'s. After incubating the virus with these extracts, methanol extracts of **1,7** and **19** reduced the infective titer at low levels ranged from 10^{-1} to $10^{-1.5}$ (Table IV). Whereas the inhibitory effect of **19** was not repeated in a dose related manner. The other extract (water extracts of **19** and **20**) did not show an obvious inhibitory effect against HIV 1.

Discussion

In the chemotherapy of HIV infections, a variety of drugs that inhibit different stages of HIV replication cycle are needed to reduce the risk of drug resistance and side effects. We have investigated anti-HIV effects of natural products from China, Indonesia, Panama and Sri Lanka to discover new compounds against HIV-1. We have reported that various plants such as *Pogostemon heynaenus*, *Jatropha curcas*,[5] *Swietenia mahoagoni* L.[6] and Shikon (Lithospermi radix)[7] inhibited the replication of HIV-1.

In this report, We screened 41 Egyptian folk medicinal plant extracts for anti-HIV-1 activity. Among these, six plants were shown to have an inhibitory effect at concentrations below cell toxicity. As the results of a more detailed test of these extracts, we found that *Croton tiglium* L. (**19**) had especially strong anti-HIV-1 activity (Table II). Moreover, this extract suppressed giant cell formation in co-cultures of HIV-infected and non-infected MOLT-4 cells (Table III), suggesting that **19** can inhibit virus adsorption or fusion, as giant cell formation depends on the interaction of HIV envelope protein with virus receptors on the cell surface[6,8,10] In addition, we found that the methanol extract of **19** inactivated the virus immediately by 10^{-1} (Table IV). To determine the precise mechanism, we investigated whether these extracts may directly interact with the viral glycoprotein gp 120 and/or CD4+cell, using 0.5 β and anti-Leu3a monoclonal antibody (mAB), respectively. However, all of these extracts, including **19**, did not interfere with the binding of mAB (data not shown), indicating that these extracts do not directly bind to the CD4 receptor or gp 120. Moreover, in these six plants, including **19**, an inhibitory effect on reverse transcriptase[11] and protease activity of HIV-1 was not shown (data not shown). In conclusion, although a detailed mechanism is not clear, the prevention of virus adsorption or fusion and virus inactivation may be the main process for anti-HIV-1 properties of the **19**. Although active constituents were not isolated and identified in this study, it is well known that the seed of *Croton tiglium* L. includes croton oil, and the principal ingredient, phorbol ester, was reported to have an inhibitory effect on HIV-1.[12] It is possible this component might also prevent the replication of HIV-1.

Although the inhibitory concentration was higher than **19**, water extracts of *Bassia muricata* (L.) MURR. (**9**) and *Centaurea scoparia* L. (**14**) also inhibited HIV-1-induced giant cell formation. These extracts also might suppress virus-cell binding or fusion. The extracts of *Abrus precatorius* L. (**1**) and *Artemisia absinthium* L. (**7**) did not prevent giant cell formation, but they had the weak direct effect against HIV-1 as in **19**.

As mentioned, none of these six plants showded any inhibitory effect on the reverse transcriptase[11] and protenese activity (data not shown) of HIV-1. These finding suggests that **1,7** and *Datura stramonium* L. (**20**) had an alternative action mechanism of the anti-HIV activity.

Several laboratories have launched an extensive screening to identify potential anti-HIV agents in natural sources[13,14] We have also reported anti-HIV-1 properties of some compounds from natural materials.[1,7,15,16] In this investigation, some active compounds with unknown action mechanism were found out. We expected that our further anti-HIV screening will result in the discovery of novel seeds for clinically-useful anti-AIDS drug with a new action mechanism of anti-HIV and fewer side effects.

和文抄録

41種類のエジプト民間薬メタノール抽出物と水抽出

物について *in vitro* における抗HIV-1活性を調べたところ，*Abrus precatorius* L., *Artemisia absinthium* L., *Croton tigrium* L., *Datura stramonium* L. のメタノール抽出物および *Bassia muricata* (L.) MURR, *Centaurea scoparia* L, *Croton tiglium* L., *Datura stramonium* L. の水抽出物に強い抗HIV-1活性を認めた。MT-4細胞におけるHIV-1によるCPE阻止濃度（IC_{50}）はそれぞれ2.0, 9.8, 0.025, 4.1, 135, 46.2, 2.0, 90 μg/mlであった。さらに *Croton tiglium* L. は感染細胞と非感染細胞の混合培養においてみられる巨細胞形成をも強く抑制したことから，抗HIV-1作用機序の一つとしてウイルスと細胞の結合を阻害することが示唆された。また，*Abrus precatorius* L., *Artemisia absinthium* L., *Croton tiglium* L. においては，ウイルスへ直接作用して感染性を低下させることがわかった。しかし，これら6種の植物から得られた8つの抽出物には，HIV-1の逆転写酵素およびプロテアーゼに対する阻害作用はなかった。

References

1) Mitsuya, H., Weinhold, K. J., Furman, P. A., St. Clair, M. H., Lehrman, S. N., Gallo, R. C., Bolognesi, D., Barry, D. W. and Broder, S. : 3' Azido 3' deoxythymidine (BW A509U) : an antiviral agent that inhibits the infectivity and cytopathic effect of human T lymphotropic virus type III/lymphadenopathy associated virus in vitro. *Proc. Natl. Acad. Sci. USA* **82**, 7096-7100, 1985.

2) Yarchoan, R., Mitsuya, H., Thomas R., V., Pluda, J., M., Hartman, N., R., Perno, C. F., Marczyk, K., S., Allain, J. P., Johns, D., G. and Broder, S. : In vitro activity against HIV and favorable toxicity profile of 2', 3' dideoxyinosine. *Science* 245, 412-415, 1989.

3) Larder, B., A., Darby, G. and Richman, D., D. : HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* **243**, 1731-1734, 1989.

4) Yamasaki, K., Otake, T., Mori, H., Morimoto, M., Ueba, N., Kurokawa Y., Shiota, K. and Yuge, T. : Screening test of crude drugs extract on anti HIV activity. *Yakugaku Zasshi* **113**, 818-824, 1993.

5) Otake, T., Mori, H., Morimoto, M., Ueba, N., Kusumoto, I., T., Lim, Y., A., Miyashiro, H., Hattori, M., Namba, T., Gupta, M., P. and Correa, M. : Anti human immunodeficincy virus activity of some tropical medicinal plants. *J. Traditional Med.* **11**, 188-193, 1994.

6) Otake, T., Mori, H., Motimoto, M., Ueba, N., Sutardjio, S., Kusumoto, I., T., Hattori, M. and Namba, T. : Screening of Indonesian plant extracts for anti human immunodeficiency virus type 1 (HIV-1) activity. *Phytherapy Res.* **9**, 6-10, 1995.

7) Ueba, N., Otake, T., Yamasaki, K., Mori, H., and Morimoto, M. : Anti HIV-1 activities of crude drug, Shikon (Lithospermi Radix), in vitro. *Nippon Rinsho* **51**, 207-212, 1993.

8) Otake, T., Miyano, K., Mori, H., Morimoto, M., Ueda, N., Kunita, N., Nakashima, H. and Kurimura, T : Anti HIV-1 activity of sulfated amphotericin B in vitro. *Anticiral Res.* **16**, 243-255, 1991.

9) Tochikura, T. S., Nakashima, H., Tanabe, A. and Yamamoto, N. : Human immunodeficiency virus (HIV) induced cell fusion : Quantification and its application for the simple and rapid screening of anti-HIV substances in vitro. *Virology* **164**, 542-546, 1988.

10) Mitsuya, H., Looney, D. J., Kuno, S., Ueno, R., Wong-Staal, F. and Broder, S. : Dextran sulfate suppression of viruses in the HIV family : inhibition of virion binding to CD4+cells. *Science*, **240**, 646-649, 1988.

11) El Mekkawy, S., Meselhy, M., R., Kusumoto, I., T., Kadota, S., Hattori, M. and Namba, T. : Inhibitory effects of Egyptian folk medicines on human immunodeficiency virus (HIV) reverse transcriptase. *Chem. Pharm. Bull.* **43**, 641-648, 1995.

12) Gustafson, K., R., Cardellina II, J., H., Mcmahon, J., B., Gulakowski, R., J., Ishitoya, J., Szallasi, Z., Lewin, N., E., Blumberg, P., M., Weislow, O., S., Beutler, J., A., Buckheit, Jr., R., W., Cragg, G., M., Cox, P., A., Bader, J., P., and Boyd, M., R. : A nonpromoting phorbol from the Samoan medical plant *Homalanthus nutans* inhibits cell killing by HIV-1. *J. Med. Chem.* **35**, 1978-1986, 1992.

13) Chang, R., S. and Yeung, H., W. : Inhibition of growth of human immunodeficiency virus in vitro by crude extract of Chinese medicinal herbs. *Antiviral Res.* **9**, 163-176, 1988.

14) Yao, X. J., Wainberg, M., A. and Parmiak., M., A. : Mechanism of inhibition of HIV-1 infection in vitro by purified extracts of *Prunella vulgaris*. *Virology* **187**, 56-62, 1992.

15) Morimoto, M., Mori, H., Otake, T., Ueba, N., Kunita, N., Niwa, M., Murakami, T. and Iwanaga, S. : Inhibitroy effect of Tachyplesin I on the proliferation of human immunodeficiency virus in vitro. *Chemotherapy* **37**, 206-211, 1991.

16) Mori, H., Otake, T., Morimoto, M., Ueba, N., Kunita, Nakagami, T., Yamasaki, N. and Taji, S. : In vitro anti human immunodeficiency virus type 1 activity of Biliverdin, a bile pigment. *Jpn. J. Cancer Res.* **82**, 755-757, 1991.