**Natural Product Sciences**
2(2) : 130 ~ 136 (1996)

# Antiviral Effects of Natural Products on the Inhibition of Hepatitis B Virus DNA Replication in 2.2.15 Cell Culture System

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**Abstract** – Evaluation of plant extracts that might inhibit hepatitis B virus (HBV) replication was performed to find potent anti-HBV agents. Eighty-five species of plants from forty-three families were tested for their anti-HBV activities using HBV-producing HepG2-derived 2.2.15 cells. The anti-HBV activity of plant extracts was measured by slot blot hybridization technique and cytotoxicity was determined by crystal violet staining procedure. All plants were extracted with methanol and the extracts were partitioned into n-hexane, ethyl acetate and aqueous layer. The ethyl acetate fractions of *Rhus verniciflua* (stem: $EC_{50}$, 8.2 μg/ml; $CC_{50}$, 9.4 μg/ml), *Gastrodia elata* (root: $EC_{50}$, 17.7 μg/ml; $CC_{50}$, >20 μg/ml), *Raphanus sativus* (seeds: $EC_{50}$, 17.3 μg/ml; $CC_{50}$, >20 μg/ml), and *Angelica gigas* (root: $EC_{50}$, 8.3 μg/ml; $CC_{50}$, 15.6 μg/ml) revealed the anti-HBV activity in 2.2.15 cell culture system and these fractions are under the process of further sequential fractionation by column chromatography to find the active principles against HBV.

**Key words** – Hepatitis B virus (HBV), anti-HBV agent, 2.2.15 cells, slot blot hybridization.

## Introduction

Human hepatitis B virus (HBV) belongs to a group of hapadnaviruses, which contains a circular DNA that is partially single stranded. HBV infection provokes the public health problem of importance in the world, especially in Far-East Asia and tropical Africa. Moreover, HBV plays an important role in the development of chronic hepatitis and hepatocellular carcinoma (HCC) (Hino *et al.*, 1986; Korba *et al.*, 1989; Huang and Chisari, 1995; Takenaka *et al.*, 1995).

Even though the clinical manifestations, epidemioloy, and pathology of HBV infection are well established, the development of anti-HBV therapeutic agents has been hampered by the lack of suitable *in vitro* culture system in which HBV can be effectively propagated. Recently, an *in vitro* screening system using HepG2-derived HBV-producing cell line (2.2.15) has been developed as a model system for screening of antiviral agents against HBV (Sells *et al.*, 1988; Korba and

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Milman, 1991; Korba and Gerin, 1992). In this system, extracellular levels of viral DNA and intracellular viral DNA replication intermediates were used as reliable markers for hepadnaviral DNA replication, which could be analyzed in a quantitative manner by blot hybridization technique (Sells *et al.*, 1988; Korba and Gerin, 1992).

Several types of antiviral agents against chronic HBV infection have been reported (Schalm *et al.*, 1995; Ponzetto *et al.*, 1991; Ruiz-moreno, 1995; McMillan *et al.*, 1995). It has long been known that interferon-α inhibits HBV replication by blocking some steps in the pregenome RNA-primed assembly of core particles (Hayash and Koike, 1989; Tur-kaspa *et al.*, 1990; Hayash and Koike, 1989; Sarin *et al.*, 1996) and nucleoside analogues inhibit DNA polymerase as well as reverse transcriptase activities (Ueda *et al.*, 1989; Nordenfelt, 1987; Alexander *et al.*, 1987; Kruining *et al.*, 1995). The nucleoside analogues such as (-)-β-L-2', 3'-dideoxy-3'-thiacytidine (3TC; lamivudine), (-)-β-L-2',3'-dideoxy-5-fluoro-3'-thiacytidine (β-L-FTC) and 2'-fluoro-5'-methyl-β-L-arabinofuranosyluracil (L-FMAU) are leading anti-HBV oxathiolane nucleoside analogues with low toxicities and potent therapeutic activities against HBV in human (Schalm *et al.*, 1995; Kruining *et al.*, 1995; Schinazi *et al.*, 1994; Chu *et al.*, 1995). In order to investigate the active principles that might inhibit HBV DNA replication, 174 plant fractions were evaluated with 2.2.15 cell culture stystem by measuring total viral DNA replicated.

## Experimental

**Plant materials** – Plant materials were purchased from a herb market in Seoul and voucher specimens have been deposited at Herbarium of Natural Products Research Institute, Seoul National University, Seoul, Korea. Dried plants were sliced, and then extracted three times with methanol at room temperature. The extracts were concentrated under reduced pressure at 40 ℃ and the concentrated methanol extracts were partitioned into n-hexane, ethyl acetate, and water layers.

**Cell culture** – HBV-producing 2.2.15 cells, transfected with a plasmid containing multiple copies of the HBV genone (Sells *et al.*, 1987), were kindly donated by Dr. B.E. Korba (Georgetown University, Rockville, MD, USA). Cells were maintained in RPMI-1640 medium containing 5% fetal bovine serum (FBS), 2 mM glutamine, 50 μg/ml gentamicin sulfate, 350 μg/ml G418 (Gibco, Inc, Grand Island, NY) at 37 ℃ (5% $CO_2$, 100% humidity).

**Anti-HBV assay** – Cultured 2.2.15 cells were seeded into 24-well microtiter plates (Corning Costar Co., USA) at a density of $20 \times 10^4$ cells/well in 0.5 ml medium and incubate for 6 days with plant extracts (dissolved in DMSO) at 37 ℃, 5% $CO_2$, 100% humidity, and an additional 2 days of incubation was performed. During the 6 days of treatment periods, test samples were added at 0, 2nd and 4th day with fresh medium and fresh culture medium was replaced at 6th day. At 8th day, the culture medium was collected and each plate was tested for cell survival using a crystal violet staining method. The collected medium was stored at -70 ℃ for later analysis of extracellular viral DNA.

**DNA extraction and slot blot hybridization** – HBV DNA was extracted from culture medium and the extracellular viral DNA was analyzed by a slot blot hybridization technique (Korba and Milman, 1991). For the analysis of extracellular HBV DNA, 0.4 ml of culture medium was incubated for 20 min at 25 ℃ in 1 M NaOH/10 x SSC (1 SSC is 0.15M NaCl/0.015M Sodium Citrate, pH 7.2) and then directly applied to nylon membrane presoaked ine 20 x SSC using a Bio-Dot SF apparatus (Bio-Rad, USA). Samples were neutralized by washing twice with 0.5 ml of 1M Tris-HCl (pH 7.2)/2M NaCl and

once with 0.5 ml of 20×SSC. Filters were then rinsed in 2 x SSC and dried in oven (55 ℃). The dried membranes were exposed to ultraviolet irradiation (0.15 J/cm$^2$) using the CL-100 ultraviolet cross linker (UVP Inc., USA). Hybridization was performed in hybridization incubator Model-400 (Robbins Scientific Co., USA) at 42 ℃ for 20 hours with HBV DNA fragment radiolabelled by nick-translation with [α-$^{32}$P]dGTP. The probe used for the hybridization analysis was gel-purifided, 3.2kb EcoRI HBV DNA fragment from pEco63 plasmid purchased fom American Type Culture Collection, USA. The anti-HBV activities of treated samples were determined by comparisons with the control group (DMSO) using a bio-imaging analyzer BAS-1500 (Fuji Photo Film Co., Ltd., Japan). $EC_{50}$ values (50% effective concentration: test compound concentration which induces a 50% decrease in the levels of extracellular HBV DNA in treated versus untreated control cells) were calculated by a linear regression analysis by using TableCurve program (Jandel Scientific Co., USA). In each experiment, DMSO solution (final 0.5%) was used as a negative control and lamivudine [(-)-β-L-2',3'-dideoxy-3'-thiacytidine] and ddC (2',3'-dideoxycytidine) were used as posi- tive controls.

**Cytotoxicity test** – Plant extracts were evaluated for their cytotoxic effect by measuring the cell survival at the end of the each experiment using the crystal violet staining method (Holobaugh and McChesney, 1990). The cells (20×10$^4$ cells/well) were seeded into 24-well microtiter plates and treated with samples (in 0.5 ml culture medium/well) as described above. At 8th day, culture medium was removed and 0.2 ml of 2% ethanol solution containing 0.2% crystal violet dye was added to each well. After incubation for 10 min, free crystal violet dye was removed by washing with PBS 3 times and the plates were air-dried. The bound dye was solubilized by the addition of 50% eth- anol solution containing 0.5% SDS. After gentle shaking for 1hr, the absorbance at 610 nm was measured using a micro plate reader THERMO$_{max}$ (Molecular Devices Co., USA). $CC_{50}$ values (50% cytotoxic concentration) were calculated by a linear regression analysis using TableCurve program (Jandel Scientific Co., USA) and TI values (Therapeutic Index) were calculated as follows.

$$TI=\frac{CC_{50}}{EC_{50}}$$

TI: Therapeutic Index
$EC_{50}$: 50% Effective concentration
$CC_{50}$: 50% Cytotoxicity

## Results and Disscussion

An *in vitro* system for assessing the effect of antiviral agents on HBV DNA replication was used for the evaluation of plant extracts by analyzing the amount of HBV DNA from culture media using slot blot hybridization and cytotoxicity measurement at 8th day after treatment. The results were summarized in Table 1 for anti-HBV activity in 2.2.15 cell culture system. The ethyl acetate fractions of *Rhus verniciflua* (stem: $EC_{50}$, 8.2 μg/ml; $CC_{50}$, 9.4 μg/ml), *Gastrodia elata* (root: $EC_{50}$, 17.7 μg/ml; $CC_{50}$, >20 μg/ml), *Raphanus sativus* (seeds: $EC_{50}$, 17.3 μg/ml; $CC_{50}$, >20 μg/ml) and *Angelica gigas* (root: $EC_{50}$, 8.3 μg/ml; $CC_{50}$, 15.6 μg/ml) revealed dose dependent inhibitory effects against HBV DNA replication in this culture system (Fig. 1). *Rhus verniciflua* and *Angelica gigas* shows the most effective inhibition against HBV replication on the basis of measurements of their reduction of HBV DNA, however, these plant fractions revealed some cytotoxic effects (Table 1 and Fig. 2). The ethyl acetate fractions of *Raphanus sativus* and *Gastrodia elata* show inhibitory activity on HBV DNA replication in 2.2.15 cell culture system without significant cytotoxicity (Table 1 and Fig. 2). In case of ethyl acetate fraction of *Poncirus trifoliata*, the antiviral effect is due to its cytotoxic activity in cell culture system. The ethyl ace-

**Table 1.** Effects of plant extracts against HBV DNA replication in 2.2.15 cells

| Plant name/family name | Plantparts | HBV activity | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ethyl acetate fraction | | | Aqueous fraction | | |
| | | $EC_{50}$ (μg/ml) | $CC_{50}$ (μg/ml) | TI | $EC_{50}$ (μg/ml) | $CC_{50}$ (μg/ml) | TI |
| *Acanthopanax senticosus*/Araliaceae | | Rb | >20 | >20 | - | >20 | >20 |
| *Aconitum kusnezoffii*/Ranunculaceae | | Rt | >20 | >20 | - | >20 | >20 |
| *Akebia quinata*/Lardizabalaceae | | Tu | >20 | >20 | - | >20 | >20 |
| *Albizzia julibrissin*/Leguminosae | | Bk | >20 | >20 | - | >20 | 13.3 |
| *Amomum cardamomum*/Zingiberaceae | | Sd | >20 | >20 | - | >20 | >20 |
| *Anemarrhena asphodeloides*/Liliaceae | | Rt | >20 | 13.5 | <0.7 | >20 | >20 |
| *Angelica dahurica*/Umbelliferae | | Rt | >20 | 16.7 | <0.8 | >20 | >20 |
| *Angelica gigas*/Umbelliferae | | Rt | 8.3 | 15.6 | 1.9 | >20 | >20 |
| *Angelica tenuissima*/Umbelliferae | | Rt | >20 | >20 | - | >20 | >20 |
| *Artemisia capillaris*/Compositae | | St, Lf | >20 | >20 | - | >20 | >20 |
| *Asparagus cochinchinensis*/Liliaceae | | Bk | >20 | >20 | - | >20 | >20 |
| *Atractylodes japonica*/Compositae | | Rt | >20 | >20 | - | >20 | >20 |
| *Benicasa cerifera*/Cucurbitaeae | | Fr | >20 | >20 | - | >20 | >20 |
| *Bupleurum chinense*/Umbelliferae | | Rt | >20 | >20 | - | >20 | >20 |
| *Bupleurum falcatum*/Umbelliferae | | Rt | >20 | >20 | - | >20 | >20 |
| *Cassia tora*/Leguminosae | | Sd | >20 | >20 | - | >20 | >20 |
| *Chaenomeles sinensis*/Rosaceae | | Sd | >20 | >20 | - | >20 | >20 |
| *Chrysanthemum sibiricum*/Compositae | | St, Lf | >20 | >20 | - | >20 | >20 |
| *Chrysanthemum sinese*/Compositae | | Fl | >20 | >20 | - | >20 | >20 |
| *Cibotium barometz*/Polypodiaceae | | Ap | >20 | >20 | - | >20 | >20 |
| *Cimicifuga heracleifolia*/Ranunculaceae | | Rt | >20 | >20 | - | >20 | >20 |
| *Citrus unshiu*/Rutaceae | | Bk | >20 | >20 | - | >20 | >20 |
| *Clematis chinensis*/Ranunculaceae | Mentha | Rt | >20 | >20 | - | >20 | >20 |
| *Cnidium officinale*/Umbelliferae | arvensis/ | Rt | >20 | >20 | - | >20 | >20 |
| *Cocculus trilobus*/Menispermaceae | Labiatae | Rt | >20 | 14.5 | <0.7 | >20 | >20 |
| *Codonopsis pilosula*/Campanulaceae | | Fr | >20 | >20 | - | >20 | >20 |
| *Crataegus pinnatifida*/Rosaceae | | Fr | >20 | >20 | - | >20 | >20 |
| *Cuscuta australia*/Convolvulaceae | | Sd | >20 | >20 | - | >20 | >20 |
| *Cyperus rotundus*/Cyperaceae | | Rt | >20 | >20 | - | >20 | >20 |
| *Dianthus chinensis*/Caryophyllaceae | | Wp | >20 | >20 | - | >20 | 18.5 |
| *Epimedium koreanum*/Berberidaceae | | Lf, St | >20 | >20 | - | >20 | >20 |
| *Eucommia ulmoides*/Eucommiaceae | | Bk | >20 | >20 | - | >20 | >20 |
| *Fritillaria verticillata*/Liliaceae | | Wp | >20 | >20 | - | >20 | >20 |
| *Gastrodia elata*/Orchidacedae | | Rt | 17.7 | >20 | 1.1 | >20 | >20 |
| *Gentiana scabra*/Gentianaceae | | Rt | >20 | >20 | - | >20 | >20 |
| *Gleditsia officinalis*/Leguminosae | | Sd | >20 | 12.6 | <0.6 | >20 | >20 |
| *Hydnocarpus anthelmintica*/Flacourtiaceae | | Sd | >20 | >20 | - | >20 | >20 |
| *Kalopanax pictum*/Araliaceae | | Bk | >20 | >20 | - | >20 | >20 |
| *Leonurus sibiricus*/Labiatae | | Lf,St | >20 | 13.0 | <0.7 | >20 | >20 |
| *Ligusticum delavayi*/Umbelliferae | | Rt | >20 | >20 | - | >20 | >20 |
| *Ligustrum japonicum*/Oleaceae | | Fr | >20 | >20 | - | >20 | >20 |
| *Liriope graminifolia*/Liliaceae | | Rt | >20 | >20 | - | >20 | >20 |
| *Loncicera japonica*/Caprifoliaceae | | Fl | >20 | >20 | - | >20 | >20 |
| *Lycium chinense*/Solanaceae | | Fr | >20 | >20 | - | >20 | >20 |
| *Lycium chinense*/Solanaceae | | Sd | >20 | >20 | - | >20 | >20 |
| *Machilus thunbergii*/Lauraceae | | Bk | >20 | >20 | - | >20 | >20 |
| *Malva verticillata*/Malvaceae | | Sd | >20 | >20 | - | >20 | >20 |

**Table 1.** Continued.

| Plant name/family name | Plantparts | HBV activity | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ethyl acetate fraction | | | Aqueous fraction | | |
| | | $EC_{50}$ (μg/ml) | $CC_{50}$ (μg/ml) | TI | $EC_{50}$ (μg/ml) | $CC_{50}$ (μg/ml) | TI |
| *Mentha arvensis*/Labiatae | Ap | >20 | 13.0 | <0.7 | >20 | >20 | - |
| *Nepeta japonica*/Labiatae | Ap | >20 | >2 | - | >20 | >20 | - |
| *Panax ginseng*/Araliaceae | Rt | >20 | >20 | - | >20 | >20 | - |
| *Perilla sikokiana*/Labiatae | Lf | >20 | >20 | - | >20 | >20 | - |
| *Perilla sikokiana*/Labiatae | Sd | >20 | >20 | - | >20 | >20 | - |
| *Peucedanum japonicum*/Umbelliferae | Rt | >20 | >20 | - | >20 | >20 | - |
| *Phellodendron amurense*/Rutaceae | Bk | >20 | >20 | - | >20 | >20 | - |
| *Phlomis umbrosa*/Labiatae | Rt | >20 | >20 | - | >20 | >20 | - |
| *Phyllostachys nigra*/Gramineae | St | >20 | >20 | - | >20 | >20 | - |
| *Pinellia ternata*/Araceae | Tu | >20 | >20 | - | >20 | >20 | - |
| *Plantago asiatica*/Plantaginaceae | Sd | >20 | >20 | - | >20 | >20 | - |
| *Polygonum cuspidata*/Polygonaceae | Rt | >20 | 11.0 | <0.6 | >20 | >20 | - |
| *Poncirus trifoliata*/Rutaceae | Fr | 15.3 | 11.9 | 0.8 | >20 | >20 | - |
| *Poria cocos*/Polyporaceae | Rb | >20 | >20 | - | >20 | >20 | - |
| *Pueraria thunbergiana*/Leguminosae | Fl | >20 | >20 | - | >20 | >20 | - |
| *Pueraria Thunbergiana*/Leguminosae | Rt | >20 | >20 | - | >20 | >20 | - |
| *Pulsatilla chinensis*/Ranunculaceae | Rt | >20 | 14.5 | <0.7 | >20 | >20 | - |
| *Raphanus sativus*/Cruciferae | Sd | 17.3 | >20 | >1.2 | >20 | >20 | - |
| *Rehmania glutinosa*/Scrophulariaceae | Rt | >20 | 10.0 | <0.5 | >20 | >20 | - |
| *Rheum palmatum*/Polygonaceae | Rt | >20 | >20 | - | >20 | >20 | - |
| *Rhus javanica*/Anacardiaceae | Lf | >20 | 12.0 | <0.6 | >20 | 18.7 | <0.9 |
| *Rhus verniciflua*/Anacardiaceae | St | 8.2 | 9.4 | 1.1 | >20 | >20 | - |
| *Salvia miltiorrhiza*/Labiatae | Rt | >20 | >20 | - | >20 | >20 | - |
| *Santalum album*/Santalaceae | St | >20 | >20 | - | >20 | >20 | - |
| *Schizandra chinensis*/Magnoliaceae | Fr | >20 | >20 | - | >20 | >20 | - |
| *Scopolia japonica*/Solanaceae | Rt | >20 | >20 | - | >20 | >20 | - |
| *Scrophularia ningpoensis*/Scrophulariaceae | Rt | >20 | >20 | - | >20 | >20 | - |
| *Siegesbeckia pubescens*/Compositae | Wp | >20 | >20 | - | >20 | >20 | - |
| *Sinapis alba*/Cruciferae | Sd | >20 | >20 | - | >20 | >20 | - |
| *Smilax china*/Liliaceae | Rt | >20 | >20 | - | >20 | >20 | - |
| *Sophora flavescens*/Leguminosae | Fl | >20 | >20 | - | >20 | >20 | - |
| *Sophora flavescens*/Leguminosae | Rt | >20 | 12.5 | <0.6 | >20 | >20 | - |
| *Sparganium stoloniferum*/Sparganiaceae | Rt | >20 | >20 | - | >20 | >20 | - |
| *Strychnos ignatii*/Loganiaceae | Sd | >20 | >20 | - | >20 | >20 | - |
| *Taraxacum platycarpum*/Compositae | Rt | >20 | >20 | - | >20 | >20 | - |
| *Trichosanthes kirilowii*/Cucurbitaceae | Sd | >20 | >20 | - | >20 | >20 | - |
| *Triticum sativum*/Gramineae | Sd | >20 | >20 | - | >20 | >20 | - |
| *Typha orientalis*/Typhaceae | Fl | >20 | >20 | - | >20 | >20 | - |
| *Vitex rotundifolia*/Verbenaceae | Fr | >20 | 17.5 | <0.9 | >20 | >20 | - |
| *Xanthium strumarium*/Compositae | Fr | >20 | >20 | - | >20 | >20 | - |
| *Zanthoxylum bungeanum*/Rutaceae | Bk | >20 | >20 | - | >20 | >20 | - |

Abbreviations: Root bark (Rb), Root (Rt), Tuber (Tu), Bark (Bk), Fruits (Fr), Seeds (Sd), Stem (St), Leaf (Lf), Flower (Fl), Aerial parts (Ap) and Whole plants (Wp). TI (Therapeutic Index)=$CC_{50}/EC_{50}$

tate fractions of *Anemarrhena asphodeloides* (root), *Angelica dahurica* (root), *Cocculus trilobus* (root), *Gleditsia officinalis* (seeds), *Leonurus sibiricus* (leef and steem), *Mentha arvensis* (aerial parts), *Pulsatilla chinensis* (root), *Rehmania glutinosa* (root), *Rhus jav-*

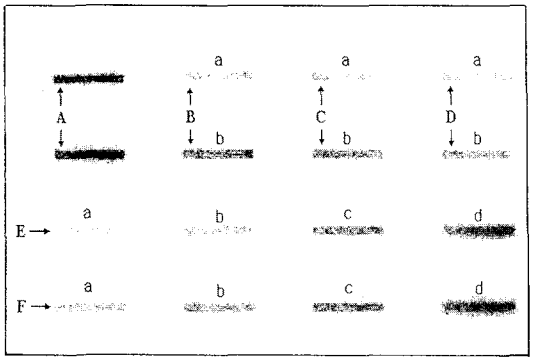


**Fig. 1.** Slot blot hybridization for the inhibitory effects against HBV DNA replication in 2.2.15 cells. DMSO (A), *Raphanus sativus* (B: a, 20 μg/ml; b, 4 μg/ml), *Gastrodia elata* (C: a, 20 μg/ml; b, 4 μg/ml), *Angelica gigas* (D: a, 20 μg/ml; b, 4 μg/ml), lamivudine [(-)-β-L-2',3'-dideoxy-3'-thiacytidine] (E: a, 2.5 μg/ml; b, 0.5 μg/ml; c, 0.1 μg/ml; d, 0.02 μg/ml), ddC (2',3'-dideoxycytidine) (F: a, 20 μg/ml; b, 4 μg/ml; c, 0.8 μg/ml; d, 0.16 μg/ml).

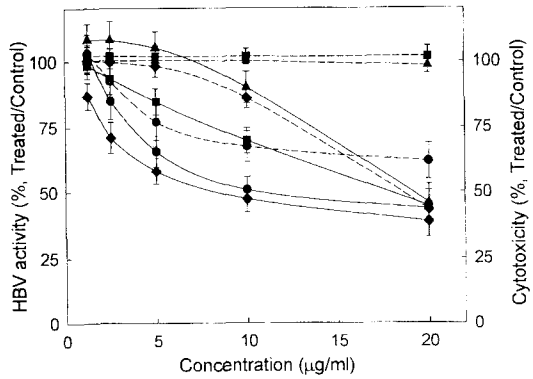


**Fig. 2.** Anti-HBV activity and cytotoxicity of ddC, *Raphanus sativus*, *Gasstrodia elata* and *Angelica gigas* in 2.2.15 cells. Curves represent means of antiviral activities and cytotoxic activities±standard errors. ●, ddC; ▲, *Raphanus sativus*; ■, *Gasstrodia elata*; ◆, *Angelica gigas*; ——, antiviral activity; --------, cytotoxic activity.

**Table 2.** Inhibitory effects of lamivudine and ddC against the extracellular HBV DNA replication in 2.2.15 cells.

| Anti-HBV agent | Effect | | |
|---|---|---|---|
| | $EC_{50}$[a](μg/ml) | $CC_{50}$[b](μg/ml) | TI[c] |
| Lamivudine[c] | 0.4 | >20 | >50 |
| ddC[c] | 9.9 | >20 | >2.0 |

[a]$EC_{50}$, 50% effective concentration. [b]$CC_{50}$, 50% cytotoxic concentration. [c]TI (Therapeutic Index)=$CC_{50}/EC_{50}$. Means from two independent experiments in duplicate were used. Lamivudine, (-)-β-L-2',3'-dideoxy-3'-thiacytidine; ddC, 2',3'-dideoxycytidine.

*anica* (leef), *Sophora flavescens* (root), *Vitex rotundifolia* (fruits) and the aqueous fractions of *Albizzia julibrissin* (bark), *Dianthus chinensis* (whole plants) and *Rhus javanica* (leef) revealed only cytotoxic effect in 2.2.15 cell culture system. Using this assay system, the inhibitory effects of both lamivudine and ddC used as positive controls were also determined (Fig. 1 and Table 2). Lamivudine ($EC_{50}$, 0.4 μg/ml; $CC_{50}$, >20 μg/ml) was 24 times more potent than ddC ($EC_{50}$, 9.9 μg/ml; $CC_{50}$, >20 μg/ml) and the ethyl acetate fractions of *Angelica gigas* (root: $EC_{50}$, 8.3 μg/ml; $CC_{50}$, 15.6 μg/ml) were more effective than ddC against HBV DNA replication on the basis of their reduction of HBV DNA.

Currently, the ethyl acetate fractions of *Gastrodia elata* (root), *Raphanus sativus* (seeds) and *Angelica gigas* (root) are under evaluation for inhibitory effects against HBV DNA replication with the use of activity-guided fractionation method with column chromatography.

## Acknowledgement

This study was supported by fund from Ministry of Science and Technology (Project No: 95-G-04-03-A-31), Korea, and fund from Miwon Co., Ltd., Korea.

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(Accepted January 17, 1997)