

Activities of Natural Plant Extracts against HIV-1

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Abstract— Anti-HIV-1 activities for the extracts (buthanol, hexane, chloroform, and water) of medicinal plants widely used in the folk medicine were evaluated for screening of anti-AIDS agents. The activities of the extracts to inhibit HIV-1 replication were also analyzed. The 50% effective concentration (EC_{50}) of inhibition activity of the p24 production for chloroform extract of *Saphora flavescens*, chloroform extract of *Herba ephedrae*, and hexane extract of *Pachyma hoelen Rumph* showed 5.8, 29.9, and 37.3 $\mu\text{g/ml}$, respectively, as good activities. Hexane extract of *Saphora flavescens*, buthanol extract of *Tulipa edulis*, hexane extracts of *Tulipa edulis*, *Herba ephedra*, and *Pachyma hoelen Rumph* in the 50% cytotoxic concentration (CC_{50}) in inhibition activities of recombinant HIV-1 RT showed 12.9, 19.5, 11.6, 12.0, and 36.8 % at concentration of 200 $\mu\text{g/ml}$, respectively, as good activities. From these results, chloroform extract of *Saphora flavescens*, chloroform extract of *Herba ephedrae*, and hexane extract of *Pachyma hoelen Rumph* were very effective against HIV-1 among all extracts tested. Therefore, we expect these plants will be a useful for anti- HIV-1 therapeutics in future.

Index Terms—Anti-HIV-1, extracts, medicinal plants

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I. INTRODUCTION

A number of laboratories are actively involved in the development of antiviral agents that interfere with HIV at different stages of viral replication [1, 2]. Unfortunately, however, their use for treatment of AIDS patients is limited due to the emergence of resistant viral, drug cross-resistance and their cellular toxicity [3, 4]. In the past 20 years, rapid progress has been made in developing natural products and chemically synthesized compounds as anti-HIV drugs [5, 6, 7]. Medicines have been developed that are targeted at many stages of the infection and replication of HIV [8, 9]. In Korea, several medicinal plant products or traditional medicines have been prescribed to treat AIDS patients [10].

In this paper, we report on the investigation of 4 organic and aqueous extracts from Korea medicinal plants for anti-HIV-1 activities. Some plant extracts significantly inhibited enzyme activities of HIV replication and HIV fusion. Therefore, these extracts were found to be candidates for further study to develop specific and potent inhibitors of HIV-1.

II. MATERIALS AND METHODS

1) Species collection

The oriental medicine and medicinal plants were given from OBM Lab., LTD, Daejeon, Korea and identified at Daejeon University. The vouchers of the species (HNU-03011~HNU-03014) have been deposited at Laboratory of Natural Products Chemistry, Hanbat National University.

2) Cells and virus

Cell lines used in this study (C8166, MT4, Hela CD4 cloned6, and HIV-1_{IIIB}/H9) were maintained in RPMI-1640 supplemented with 10% heat inactivated newborn calf serum (Gibco). The cells used in all experiments were in log-phase growth. HIV-1_{IIIB} was obtained from the culture supernatant of H9/HIV-1_{IIIB}

cells. HIV-1 A102 (NIH AIDS Research & Reference Reagent Program, US) –resistant to AZT was used for the initial infection of the MT4 cells. The 50% HIV-1 tissue culture infectious dose (TCID₅₀) of the virus preparation was determined in the relevant target cells using standard techniques.

3) Cytotoxicity assay

The cellular toxicity of extracts on MT4 cells was assessed by WST-1 method was used. The cells were incubated with 0.5 mM of WST-1 solution containing 20 mM of 1-methoxy PMS (Dojindo, Japan) at 37 °C for 2 h. The production of WST-1 formazan was measured by a microplate reader at 450 nm. The cytotoxicity of inhibitors was calculated as the relative rate of WST-1 formazan production to that of the dox-treated cells. The cytotoxic concentration that caused the reduction of viable cells by 50% (CC⁵⁰) was calculated from dose–response curve.

4) ELISA for HIV-1 p24 antigen

The effect of extracts on HIV-1 replication in vitro was measured by p24 release assay (Vironostika, BioMerieux Co., Metherland). The wells of microelisa strips ate coated with antibodies to HIV-1 p24 core antigen. Disruption buffer is added of disrupt HIV-1 virions present in test samples. All samples (100 µl in each well) were incubated at 37°C for 60 min. After washing with diluted phosphate-buffered saline (PBS), 100 µl of anti-HIV-1 (human) conjugate labeled with horseradish peroxidase (HRP) was added to each well, and incubated at 37°C for 60 min. Then 100 µl of tetramethylbenzidine substrate in urea peroxide solution was added to each well after a washing step, and the plates were incubated at room temperature for 30 min. Finally, the color reaction was stopped by adding 100 µl of 1M sulfuric acid. The absorbance of each well was read at 450 nm within 15 min by the ELISA reader. The concentration of reducing p24 antigen expression by 50% (EC₅₀) was determined from the dose–response curve.5) Protection for HIV-1 induced lytic effects

5) Inhibition assay of recombinant HIV-1 RT activity

The effect of the crude extracts on reverse transcription was tested using a non-radioactive HIV-RT colorimetric ELISA kit from Roche Diagnostics, Germany. The protocol outlined in the kit was followed using 2 ng of enzyme in a well and incubating the reaction for 2 h at 37 °C. Extracts were tested at 0.2 mg/ml.

III. RESULTS

Anti-HIV-1 activity by cytotoxicity and P24 antigen assay

The 50% cytotoxic concentration (CC₅₀) of plant extracts having activities of anti-HIV-1 was shown in Fig. 1. The CC₅₀ of plant extracts having activities of anti-HIV-1 was shown in Fig. 1A. The EC₅₀ by P24 antigen assay of SF-3, HE-3, and PH-4 showing higher inhibition on HIV-1 than the other extracts were 5.8, 29.9, and 37.3, respectively (Fig. 2).

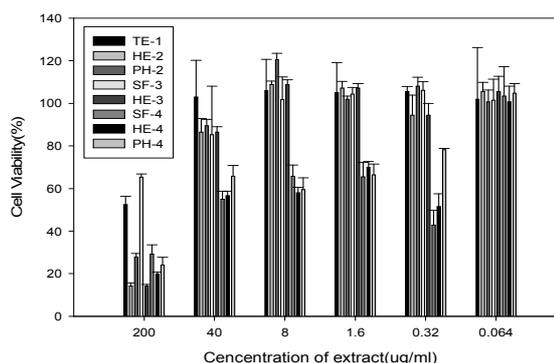


Fig. 1 Cytotoxicity of MT-4 cells treated with four extracts of medicinal plants using WST-1 assay.

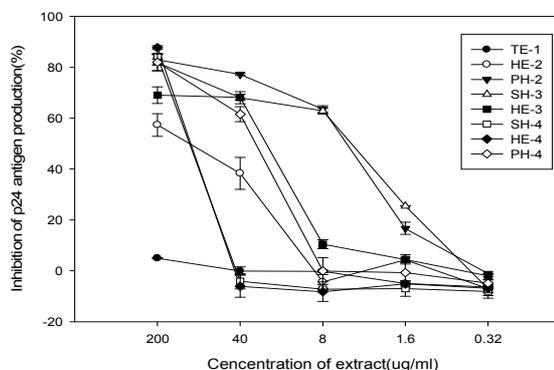


Fig. 2 Inhibition effect of HIV-1 using ELISA with p24 antigen

Inhibition of plant extract on HIV-1 RT activity

Most extracts did not inhibit the activity of recombinant HIV-1 RT. SF-4, TE-1, TE-4, HE-4, and PH-4 showed inhibition on the activity of recombinant HIV-1 RT as 12.9, 19.5, 11.6, 12.0, and 36.8 % at concentration of 200 µg/ml (Fig. 3).

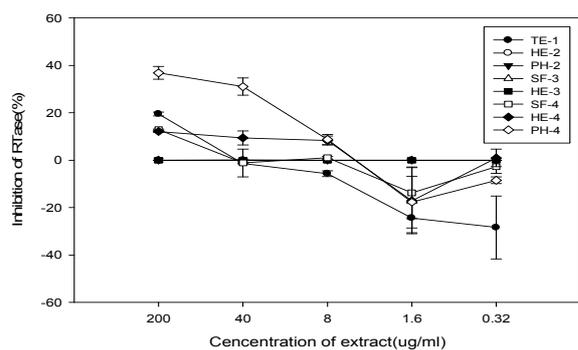


Fig. 3 Inhibition effects of HIV-1 using ELISA with RTase

IV. CONCLUSIONS

In this study, the anti-HIV-1 activities for the butanol, hexane, chloroform and water extracts of four medicinal plants widely used in the folk medicine were evaluated. Among these extracts, some extract represented prevalent anti-HIV-1 activities in various inhibition tests. The 50% effective concentration (EC_{50}) of inhibition activity of the p24 production for chloroform extract of *Saphora flavescens*, chloroform extract of *Herba ephedrae*, and hexane extract of *Pachyma hoelen Rumph* showed high activities as 5.8, 29.9, and 37.3 $\mu\text{g/ml}$, respectively. The 50% cytotoxic concentration (CC_{50}) in inhibition activities of recombinant HIV-1 RT in hexane extract of *Sophora flavescens*, butanol extract of *Tulipa edulis*, hexane extracts of *Tulipa edulis*, *Herba ephedra*, and *Pachyma hoelen Rumph* in showed 12.9, 19.5, 11.6, 12.0, and 36.8 % at concentration of 200 $\mu\text{g/ml}$, respectively, as high activities. From these results, chloroform extract of *Saphora flavescens*, chloroform extract of *Herba ephedrae*, and hexane extract of *Pachyma hoelen Rumph* were more effective against HIV-1 than any plant extracts tested. Among them, hexane extract of *Pachyma hoelen Rumph* was shown the best anti-HIV-1 activity in all test performed. Therefore, we expect these extracts of plants will be a useful as anti- HIV-1 therapeutics in future.

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