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# Anti-HIV and Antihepatotoxic Constituents from Medicinal Plant Resources

**Jong-Cheol Park, Ju-Gwon Park, Jong-Moon Hur, Young-Hee Hwang and Deuk-Young Jung**

Department of Oriental Medicine Resources, Sunchon National University, Sunchon, Jonnam 540-742 Republic of Korea, jcpark@sunchon.ac.kr

## ABSTRACT

**Medicinal plants were screened for the inhibitory effects on human immunodeficiency virus type 1 protease. Of the extracts tested, the strong inhibitory effects were observed in the acetone extracts of the pericarp of *Camellia japonica*. Camelliatannin H from the pericarp of C. japonica showed a potent inhibitory activity on HIV-1 protease. Effects of the extract and compound from leaves of *Zanthoxylum piperitum* on the enzyme activities were investigated in the liver of bromobenzene-treated rats. The methanol extract and protocatechuic acid isolated from *Z. piperitum* reduced the activity of aniline hydroxylase that increased by bromobenzene, while did not affect the activities of aminopyrin N-demethylase and glutathione S-transferase. The extract and protocatechuic acid recovered significantly the activity of epoxide hydrolase decreased by bromobenzene.**

## INTRODUCTION

The global AIDS situation has been a serious problem since this infection affects adults of working age, adolescents and children, without any effective treatment at the moment. After the first patient with AIDS was reported in 1981 in the United State, the number of AIDS patients and persons infected with its causative virus (HIV) increased abruptly all over the world. HIV has been reported as the aetiological agent of AIDS (Barre-Sinoussi *et al.*, 1982). This virus has some specific enzymes, such as reverse transcriptase, RNase H, intergrase and protease, which are necessary for its replication. Therefore, these enzymes are targets for development of anti-AIDS agents. Especially, the PR (aspartic acid protease) is responsible for the specific amino acid sequences to give functional proteins or enzymes.

Bromobenzene is a toxic industrial solvent that is known to produce centrilobular hepatic necrosis through the formation of reactive epoxides as the toxic intermediates. Bromobenzene is converted to bromobenzene 3,4-oxide by mixed function oxidase system in the liver. The electrophilic bromobenzene 3,4-oxide acts as a liver toxin (Jollow *et al.*, 1974).

Corresponding author : **Jong Cheol Park,** Department of Oriental Medicine Resources, Sunchon National University, Sunchon, Jonnam 540-742 Republic of Korea, jcpark@sunchon.ac.kr

In the course of our studies to find the anti-HIV and antihepatotoxic components in natural sources we have investigated the biological activities of *Camellia japonica* and *Zanthoxylum piperitum*. In the present study we report on the potent substance from *Camellia japonica* L. for the inhibitory effects on HIV-1 protease and the antihepatotoxic compound isolated from *Z. piperitum* in bromobenzene-treated rats.

## MATERIALS AND METHODS

### Compounds

Camelliatannins were isolated from the pericarp of *C. japonica*.. From the leaves of *Z. piperitum*, protocatechuic acid was isolated and characterized by the spectral analysis.

### HIV-protease assay

The protease inhibitory activity was determined by incubating the compound in a reaction containing protease and substrate to perform proteolytic cleavage reaction. The cleaved product was measured by reverse-phase HPLC, using a gradient of acetonitrile/0.1% trifluoroacetic acid as a mobile phase. Recombinant HIV-1 protease (purity 95% by sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was purchased from Bachem (Feinchemikalien AG, Bubendorf, Switzerland). Twenty five $\mu$L of HIV-1 PR assay buffer (Bachem, HIV protease assay kit S-1000) containing 2.5 $\mu$L of a substrate, His-Lys-Ala-Arg-Val-Leu-(p$NO_2$-Phe)-Glu-Ala-Nle-Ser-$NH_2$, was mixed with 2.5 L of a DMSO solution of test compound and then 2.5 $\mu$L of recombinant HIV-1 protease (0.175$\mu$g protein) was added to the mixture. After incubation at 37℃ for 15 min, the reaction was stopped by addition of 2.5 $\mu$L of 10% trifluoroacetic acid.

### Antihepatotoxic activity

Bromobenzene (Zampaglione *et al.*, 1973) was *i.p.* injected four times with 12 hr interval for final two days of the one week. The liver exhaustively perfused with ice-cold normal saline through the portal vein until uniformly pale and immediately removed and weighed. The cytosolic fraction was used as the enzyme sources of glutathion S-transferase. And the microsomal fraction was used for the measurement of the activities of aminopyrine N-demethylase, aniline hydroxylase and epoxide hydrolase. Glutathione S-transferase activity (Habig and Pabist, 1974) was assayed by conjugated glutathione 2,4-dinitrobenzene formation from 1-chloro-2,4-dinitrobenzene. Aminopyrine N-demethylase activity (Nash, 1953) was assayed by measuring the production of formaldehyde formed by the demethylation of aminopyrine. Aniline hydroxylase activity ( Bidlack and Lowery, 1982) was assayed by determining *p*-aminophenol formation from aniline. Epoxide hydrolase activity (Hammock and Hasegawa, 1982) was measured spectrophotometically by monitoring the rate of *trans*-stilbene oxide decreasing at 229 nm.

## RESULTS AND DISCUSSION

### Anti-HIV compound

HIV possesses some enzymes that work on viral replication, such as RNA-dependent DNA polymerase or reverse transcriptase, integrase and protease. In the first step of replication, reverse transcriptase 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 virus. The maturation of the virus takes place at the last step by viral protease, which cleavage the viral polyproteins at the specific amino acid sequences to give functional proteins or enzymes. The mature viruses bud from the cells and continuously infect other T-cells. The blocking of any of these steps in the viral life cycle is expected to stop the viral replication. Recently,

clarification on the structure and function of viral enzyme, a protease, has shown another target in HIV. Therefore HIV-protease has been a good tool for this investigation since its inhibitors may suppress the HIV-1 production from chronically infected cells (Mcquade et al., 1990; Meek et al., 1989).

The extracts of Korean medicinal plants have been studied for the inhibitory effects of HIV-1 protease. In the screening of these samples, the methanol extracts of the pericarp of *C. japonica* showed a strong inhibitory activity against HIV-1 protease. We have studied anti-HIV-protease components from the pericarp of *C. japonica* having appreciable inhibitory activity. Since *C. japonica* which have been used as a hemostasis and an astringent in Korea contains significant amounts of tannin, we assumed that inhibitory action of the methanol extract was primarily due to tannins. From the purpose of conforming this, the acetone extract which is more suitable for the isolation of tannins was carried out. The acetone extract of the percarp of *C. japonica* showed the most potent inhibitory effect among the acetone extracts of various parts of this plant. Each fractions from the acetone extract of the pericarp were tested for HIV-1 protease inhibitory effects, in which n-BuOH fraction showed the strong inhibition among the fractions, and it was further fractionated to isolate the major inhibitory substances.

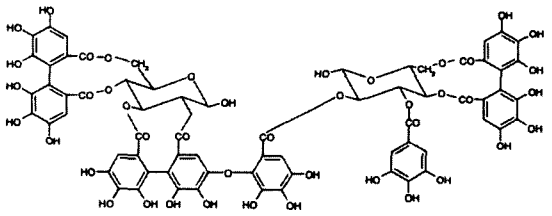
Camelliatannin H, a dimeric hydrolyzable tannin from the peicarp of *C. japonica*, showed the most potent inhibitory activity against HIV-1 protease with $IC_{50}$ value of 0.9 $\mu$M. Camelliatannins A and F, complex tannins isolated from C. japonica showed the moderate inhibitory activities.

**Antihepatotoxic compound**

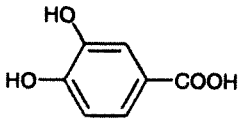
Bromobenzene is a xenobiotic liver toxin that is known to produce centrilobular hepatic necrosis through the formation of reactive epoxides as the toxic intermediates. In the metabolism of bromobenzene, the nontoxic 2,3-epoxide, which readily forms 2-bromophenol, or the toxic 3,4-epoxide are produced on oxidation by cytochrome P-450 monooxygenases. Several pathways exist that can detoxify the reactive 3,4-epoxide; rearrangement to the 4-bromophenol, hydration to the 3,4-dihydrodiol catalyzed by epoxide hydrolase, or conjugation with glutathione. When more 3,4-epoxide is produced than can readily be detoxified, cell injury increases (Levi, 1987).

Rats were orally administered daily with the methanol extract from the leaves of *Z. piperitum* and protocatechuic acid for one week prior to bromobenzene treatment. Then bromobenzene was *i.p.* injected four times with 12 hr interval for final two days after the extraction or compound treatment.

The activities of the hepatic enzymes involved in formation and metabolism of epoxides, aminopyrine N-demethylase, aniline hydroxylase, glutathione S-transferase and epoxide hydrolase were investigated. The activities of microsomal aminopyrine N-demethylase and aniline hydroxylase in liver were significantly increased by bromobenzene injection. Pretreatment of the extract of Z. piperitum or isolated component decreased aniline hydroxylase activity, an enzyme producing bromobenzene epoxide. The extract pretreatment did not show any effect on the increase of aminopyrine N-demethylase activity. Although the compound group showed a weak inhibition in this enzyme activity, the significant change was not observed. Glutathione S-transferase catalyzes the reaction of a wide variety of electrophiles with glutathione. Bromobenzene 3,4-oxide, an epoxide acting as a liver toxin, can be detoxified by glutathione S-transferase, a hepatic enzyme containing glutathione. The extract pretreatment did not change the enzyme activity increased by bromobenzene. The isolate of *Z. piperitum* showed the weak inhibition of this enzyme



**Camelliatannin H**




**Protocatechuic acid**

acitivity. Bromobenzene-oxide is also metabolized to a nontoxic bromobenzene 3,4-dihydrodiol by epoxide hydrolase. This enzyme activity of the control group showed significant decrease by bromobenzene, which was recovered by pretreatment of the extract or protecatechuic acid from *Z. piperitum*. We suggest that protocatechuic acid might prevent the hepatotoxicity by reduction of the activity of aniline hydroxylase, an epoxide-producing enzyme along with enhancement of the activity of epoxide hydrolase, an epoxide-removing enzyme.

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