Korean J. Medicinal Crop Sci. 10(4) : 284-287(2002)

Inhibitory Effects of the Extract of *Rhus verniciflua* Stokes on the Reverse Transcriptase of AIDS

Myong Jo Kim*†, Won Cheol Choi**, A. M. Barshinikov*** and A. Kobayashi****

* *College of Agricultural and Life Science, Kangwon National University, Chuncheon, 200-701, Korea*
** *Kwanghyeowon Mediacal Foundation Cancer Center, Korea*
*** *Russian N. N., Blocklhin Cancer Center, Moscow*
**** *Osaka National University, Japan*

ABSTRACT : **Four olefinic catechols, commonly referred to as urushiol were isolated from the sap of Korean *Rhus verniciflua* Stokes and had the stronger inhibitory effects on the reverse transcriptase of AIDS. The hexane extract with a inhibitory effects on reverse transcriptase was purified by silica and ODS gel column chromatography. The active compounds were identified by MS and ^{1}H-NMR as 3-[8′(Z), 11′(Z), 14′-pentadecatrienyl]catechol, 3-[8′(Z),11′(Z)-pentadecadienyl]catechol, 3-[8′(Z)-penta- decenyl]catechol, and 3-pentadecylcatechol. All of these compounds showed strong inhibitory effects on reverse transcriptase of AIDS, in which 3-pentadecylcatechol exhibited the highest activity (IC_{50} : 10.87 μg/ml).**

Key words : *Rhus vernicifera*, lacquer tree, urushiol, reverse transcriptase, AIDS

INTRODUCTION

Rhus verniciflua is a defoliating tree of the *Anacardiaceae* family. The place of origin is China and it is mainly cultivated in Southeast Asia countries such as Korea, Japan and China. The sap was *Rhus verniciflua* and has been used for industrial and medical purposes and medicinal waxes were gathered from the fruit(Lee, 1982). The durable characteristics of lacquer have been used for a long time to preserve and to varnish the furniture naturally. The sap from *Rhus verniciflua* was a type of plant secretion and was found in the secondary phloem of the stem bark. Due to enzyme reactions, lacquer contacts with air and hardens the surfaces. It forms a great 3-dimensional surface unlike other varnishes. *Rhus verniciflua* shines and doesn't change even after a long time, thus, has been used widely in Korea, China and Japan for a long time. *Rhus verniciflua* did not corroded by acid or alkaline and was durable with great heat-resistance, water-resistance, preservation and protection against insects(Mok, 1974). *Rhus verniciflua* was used not only for crafts, but also for industrial crafts, industrial applications, edible and medicinal materials. Demand would be increased rapidly with future industrial development.

In the Orient, *Rhus verniciflua* was used for medicine prescriptions as folk remedies for abnormalities in women's menstrual cycles, digestive medicine, vermicides, blood circulation and prevention of aging. In Korea, *Rhus verniciflua* was used to make the chickens- and ducks-food to use in Oriental medicine and folk medicine for preventing paralysis and high blood pressure and as invigorants (Kim, 1986). When *Rhus verniciflua* was cutted externally a white liquid (pure extract when purified) was released. When coming into contact with animal skin, this liquid sometimes caused irritation. Sensitive people have an

† Corresponding author (Phone) : 033-250-6410, E-mail : kimmjo@kangwon. ac. kr
Received 10 October, 2002 / Accepted 30 October, 2002

sin irratated reaction even with a small amount of *Rhus verniciflua*.

Most of the researches on the chemical characteristics of *Rhus verniciflua* were done with the skin irritated components and purification of *Rhus verniciflua*(Zhai et al., 1999).

Recently, there was an urgent need for preventing and curing the acquired immunodeficiency syndrome (AIDS) and human T cell leukemia (HALT). AIDS has been caused by the human immunodeficiency virus (HIV) and human T.cell leukemia also caused by the human T cell leukemia virus. These viruses have reverse transcriptase which transforms RNA into DNA. For such reason, there has been much researches for preventing AIDS by inhibiting reverse transcriptase viruses (Soriano et al., 2000; Liska et al., 1999; Tantillo et al., 1994). As a result, many chemicals having the inhibitory effects on reverse transcriptase of AIDS has been developed. For example, 3′-azido-3′-deoxy-thymidine (AZT), 2′,3′-dideoxyinosine(DDI), and 2′,3′-didehydro-3′- deoxythymidine (D4T) have been approved as AIDS remedy. However, the price of these medicines were too expensive to manufacture and there were many side effects(Larder, 1995; Schinazi et al., 1990; Lin et al., 1987). Therefore, this study was conducted to isolate the active components and to determine the inhibition effects of the extract of the Korean *Rhus verniciflua* on reverse transcriptase of AIDS

MATERIALS AND METHODS

Pharmacological activity of *Rhus verniciflua* extracts

Active fractions were conducted using *Rhus verniciflua* obtained from Hoengseong, Gangwon-do, and the pharmacological activation was studied. First, *Rhus verniciflua* including the bark was dried. *Rhus verniciflua* was extracted by methanol. After removed the methanol, the methanol extract was diluted to a volume of 1L by the addition of distilled water and 1000ml of hexane was added to separate *Rhus verniciflua* extract into a water layer and a hexane layer. The aqueous layer was further extracted with 1L of ethylacetate and butanol. Each separation was extracted to get a 13.3g hexane layer, 5.4g EtOAc layer, 3.8g BuOH layer and 32.9g water layer.

Separation and purification of active compounds of *Rhus verniciflua* extracts

Of the solvent fractionations extracted from *R. verniciflua*, active compounds were separated and purified from the hexane layer which showed the most powerful inhibitory effects on reverse transcriptase of AIDS(data not shown here). The hexane extract of 4g showing inhibitory activity was subjected to silica gel column chromatography and silica gel [(5 70cm, 7734 Merck] using a solvent gradient system from n-hexane to acetone. The active fractions (20% acetone eluate) were further purified by a silica gel column chromatography (5 cm x 70 cm, Merck 9385) eluting with a stepwise mode of ethyl acetate and n-hexane. The active fractions (20% ethyl acetate eluate) were finally purified using ODS gel column chromatography (1.65 x 100 cm, YMC GEL ODS-A) with a gradient of methanol and water. 23.4mg of the compound 1(3-[8′(Z), 11′(Z), 14′-pentadecatrienyl] catechol), 16.8mg of the compound 2(3-[8′(Z), 11′(Z)-pentadecadienyl]catechol), 243.6mg of the compound 3(3-[8′(Z)-pentadecenyl] catechol) and 1032mg of the compound 4(3-pentadecylcatechol) were isolated.

Inhibitory effects of compounds on reverse transcriptase

Inhibitory effects on reverse transcriptase were studied using four urushiol derivative chemical compounds[compound 1(3-[8′(Z), 11′(Z), 14′-pentadecatrienyl] catechol), compound 2(3-[8′(Z), 11′(Z)-pentadecadienyl]catechol), compound 3(3-[8′(Z)-penta- decenyl] catechol) and compound 4(3-pentadecylcatechol)], and epigallocatechin gallate for comparison.

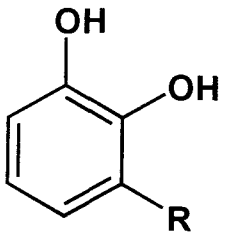
10μl of combined liquid (0.05M Tris-HCl buffer solution pH 8.0, 0.01 M DTT, 0.1 M potassium chloride, 0.0006 M magnesium chloride, 2 μg/ml Poly (A) p(dT)15, 0.37 μm ^{3}H-dTTP, 10μmdTTP, glycerol/water = 25/16), 10 of 0.000 U/ml HIV-1 reverse transcriptase liquid and 10μl of buffer solution (50mM Tris-HCl pH 8.0, 10mM DTT, 200mM potassium chloride, 50 v/v% glycerol) was added to a 30μl sample of the above chemical compounds for a total volume of 50μl. This was

mixed and separated by centrifuge, and then cultured for 30 minutes at 37℃. 20μl of EDTA was added on ice, mixed and separated with centrifugal separation. The above 50μl reaction liquid was spotted on an ion exchange cellulose filter. After drying, it was washed in 5% dihydro sodium phosphate (8 times), water (2 times), ethanol (2 times) and diethylether (2 times). The ion exchange cellulose filter was placed in a scintillator and measured with the scintillation counter. The density of the sample chemical compound was between 0.01~100μg/ml and when the inhibition rate was 50%, concentration was IC_{50}. The IC_{50} of the samples are shown in table 1 and each sample showed low toxicity.

RESULTS AND DISCUSSION

The extract of *Rhus vernicifera* was partitioned between n-hexane and water. The hexane-soluble fraction showing inhibition activity on reverse transcriptase was subjected to silica and ODS gel column chromatography. The active compounds were identified as four olefinic catechols such as 3-[8′(Z), 11′(Z), 14′-pentadecatrienyl]catechol, 3-[8′(Z), 11′(Z)- pentadecadienyl] catechol, 3-[8′(Z)-pentadecenyl] catechol, and 3-pentad- ecylcatechol (Fig. 1) by the comparison of their ^{1}H-NMR, EI-MS, IR and UV (Markiewitz et al., 1965; Du et al., 1984; Yamauchi et al., 1982; ElSohly et al., 1982).

Most components in the extract were extracted by hexane, which showed a high inhibitory activity on



1. R : $-(CH_2)_7CH=CHCH_2CH=CHCH_2CH=CH_2$
2. R : $-(CH_2)_7CH=CHCH_2CH=CH(CH_2)_2CH_3$
3. R : $-(CH_2)_7CH=CH(CH_2)_5CH_3$
4. R : $-(CH_2)_{14}CH_3$

Fig 1. Chemical structure of four urushiol derivatives isolated from the extract of *Rhus vernicifera*

reverse transcriptase (IC_{50} : 0.87μg/ml). Four urushiol derivatives isolated from hexane extracts showed high inhibitory activities on reverse transcriptase of AIDS (Table 1). Four compounds showed different activities depending on the number of double bonds in side chain. The activities (IC_{50}) of four urushiol derivatives were ranged from 0.87 to 4.01μg/ml. Of four compounds, compound 4 showed the highest activity (IC_{50} : 0.87μg/ml) and compound 1 showed the lowest activity(4.01μg/ml).

Four olefinic catechols isolated in this study are

Table 1. Inhibitory effects of compounds isolated on reverse transcriptase

(unit IC_{50} : μg/ml)

Compounds	Inhibition activity (IC_{50} : μl/ml)
Epigallocatechin gallate	0.09
3-pentadecylcatechol	0.87
3-[8′(Z)-pentadecenyl]catechol	2.32
3-[8′(Z), 11′(Z)-pentadecadienyl]catechol	1.13
3-[8′(Z), 11′(Z), 14′-pentadecatrienyl]catechol	4.01

well known irritating chemicals, commonly referred to as urushiol, from poison ivy plant and related plants (Markiewitz et al., 1965; Hill et al., 1934; Adawadkar et al. 1983). Jung et al.,(1990) analyzed the components of Korean *Rhus verniciflua*, but there were no reports on the bioactive components in the same material. So far, there was no report that urushiol derivatives had high inhibitory activities on reverse transcriptase of AIDS. This is the first report on the high inhibitory activities on reverse transcriptase of AIDS of the four urushiol derivatives. There were many reports that Korean *Rhus verniciflua* had the strong biological activities such as antioxidants(Kim et al., 1997), anticancer(Hong et al. 1999) and antifungal activities(Kim et al. 1997). It is well recognized that free radicals are critically involved in various pathogenesis and in the degenerative processes associated with aging(packer et al., 1993) Urushiol derivatives could be developed for a useful antioxidant for various applications. In Korea, chicken or duck soup containing

the bark of *Rhus verniciflua* been used as a healthy food, and the crude sap have been used as a folk medicine for the treatment of cancer patients. In addition to antioxidative, antifungal and anticancer activities of urushiol derivatives, they have other biological activities including inhibitory activities on reverse transcriptase of AIDS. Thus, urushiol derivatives may be a good source for several medicine. The in vivo test of active compounds using mouse is under investigation.

LITERATURE CITED

Lee CB (1982) Illustrated guide to Korean flora. Hyangmunsa.

Mok YS (1974) Characteristics and research on lacquer. Science and Technology. 7 : 37-41.

Zhai H, Willard P, Maibach HI (1999) Putative skin-protective formulations in preventing and/or inhibiting experimentally-produced irritant and allergic contact dermatitis. Contact Dermatitis. 41(4) : 190-192.

Soriano V, Gomes P, Heneine W, Holguin A, Doruana M, Antunes R, Mansinho K, Switzer WM, Araujo C, Shanmugam V, Lourenco H, Gonzalez-Lahoz J, Antunes F (2000) Human immunodeficiency virus type 2 (HIV-2) in Portugal : clinical spectrum, circulating subtypes, virus isolation, and plasma viral load. J Med Virol. 61(1) : 111-116.

Tantillo C, Ding J, Jacobo-Molina A, Nanni RG, Boyer PL, Hughes SH, Pauwels R, Andries K, Janssen PA, Arnold E (1994) Locations of anti-AIDS drug binding sites and resistance mutations in the three-dimensional structure of HIV-1 reverse transcriptase. Implications for mechanisms of drug inhibition and resistance. J Mol Biol. 243(3) : 369-387.

Liska V, Khimani AH, Hofmann-Lehmann R, Fink AN, Vlasak J, Ruprecht RM (1999) Viremia and AIDS in rhesus macaques after intramuscular inoculation of plasmid DNA encoding full-length SIVmac239. AIDS Res Hum Retroviruses. 15(5) : 445-450.

Larder BA (1995) Viral resistance and the selection of antiretroviral combinations. J Acquir Immune Defic Syndr Hum Retrovirol. :10 Suppl 1 : S28-33

Schinazi RF, Sommadossi JP, Saalmann V, Cannon DL, Xie MY, Hart GC, Smith GA, Hahn EF (1990) Activities of 3'-azido-3'-deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1. Antimicrob Agents Chemother. 34(6) : 1061-1067.

Lin KT, Huang SH, Kao CL, Huang KM, Yu JC, Hung TP, Chou MY, Liu WT, Fang CT, Kuo YT (1987) An autopsy-proved case of AIDS in Taiwan. Asian Pac J Allergy Immunol. 5(1) : 25-31.

Tyman J H P (1979) Non-isoprenoid pong chain phenols. Chem. Soc. Rev. 8 : 499-537.

Majima R (1922) Ber den Hauptbestandteil des Japan lacks, Ⅷ. Mitteilung : Stellung der Doppelbindungen in der Seitenkette des Urushiols und Beweisf hrung, da das Urushiol eine Mischung ist. Chem. Ber. 55B : 172-191.

Markiewitz KH, Dawson CR (1965) On the isolation of the allergenically active components of the toxic principle of poison ivy. J. Org. Chem. 30 : 1610-1613.

Hill GA, Mattacotti V, Graham WD (1934) The toxic principle of the poison ivy. Am. Chem. Soc. 56 : 2736-2738.

Adawadkar PD, Elsohly MA (1983) An urushiol derivative from poison sumac. Phytochemistry. 22 : 1280-1281.

Xiong Q, Kadota S, Tadata T, Namba T (1996) Antioxidative effects of phenylethanoids from Cistanche deserticola. Biol. Pharm. Bull. 19 : 1580-1585.

Kim WG, Kim JP, Kim CJ, Lee KH, Yoo ID (1996) Benzastatins A, B, C, and D : New free radical scavengers from Streptomyces nitrosporeus 30643. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities. J. Antibiotics 49 : 20-25.

Ohkawa H, Ohishi N, Ygi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95 : 351-358.

Du Y, Oshima R, Kumanotani J (1984) Reversed-phase liquid chromatographic separation and identification of constituents of urushiol in the sap of the lac tree, *Rhus vernicifera*. J. Chromatog. 284 : 463-473.

Yamauchi Y, Oshima R, Kumanotani J (1982) Configuration of the olefinic bonds in the heteroolefinic side-chains of Japanese lacquer urushiol. J. Chromatog. 243 : 71-84.

EISohly M, Adawadkar APD, Ma CY, Turner CE (1982) Separation and characterization of poison ivy and poison oak urushiol components. J. Nat. Prod. 45 : 532-538.

Jung DK, Song HK, Kim H (1990) the characteristics of allegic materials from Korean lacquer tree sap : Preperative analysis of lacquer tree sap. Res. Rept. RDA 33 : 675-682.

Packer L, Glazer AN (1993) In Oxygen Radicals in Biological Systems : Oxygen Radicals and Antioxidants. Academic Press, Inc., San Diego, U. S. A.

Hong DH, Han SB, Lee CW, Park SH, Jeon YJ, Kim MJ, Kwak SS, Kim HM (1999) Cytotoxicity of urushiols isolated from sap of korean lacquer tree(*Rhus verniciflua*). Arch Pharm Res. 22 : 638-641.

Kim MJ, Choi YH, Kim WG, Kwak SS (1997) Antioxidative activity of urushiol derivatives from the sap of lacquer tree (*Rhus verniciflua*). KJPR Korean J. Plant. Res. 10(3) : 227-230.

Kim MJ, Kim CJ, Kwak SS (1997) Antifungal activity of urushiol components in the sap of lacquer tree(*Rhus verniciflua*). KJPR Korean J. Plant. Res. 10(3) : 231-234.

Kenneth H, Charles M, Dawson R (1964) On the isolation of the allergenically active components of the toxic principle of poison ivy. J. Org. Chem. 30 : 1610-1613.

James C, Murphy E, Watson S, Ernest C (1983) Toxicological evaluation of poison oak urushiol and its esterified derivative. Toxicology. 26 : 135-142.