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# Antioxidative Activity of Urushiol Derivatives from the Sap of Lacquer Tree (*Rhus vernicifera* Stokes)

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## ABSTRACT

The authors isolated four olefinic catechols, commonly referred to as urushiol, from the sap of Korean lacquer tree (*Rhus vernicifera* STOKES) with stronger antioxidative activities than α-tocopherol. The hexane extract with a free radical scavenging activity 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)-pentadecenyl] catechol, and 3-pentadecylcatechol. All of these compounds showed strong free radical scavenging activities on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, in which 3-pentadecylcatechol exhibited the highest activity ($IC_{50}$: 1.2 μg/ml). They also showed a significant inhibitory activity on lipid peroxidation ($IC_{50}$: 2.1 - 3.5 μg/ml). The antioxidative activity of 3-pentadecylcatechol on DPPH radical and lipid peroxidation is approximately two times greater than that of α-tocopherol. The results suggest that the urushiol derivatives may contribute to the preservative characteristics effective against oxidative stress and could be a good source for industrial applications including a coating material.

**Key words**: *Rhus vernicifera*, lacquer tree, urushiol, antioxidant, DPPH radical, lipid peroxidation

## INTRODUCTION

For thousands of years(Onishi, 1995; Tyman, 1979) the sap of oriental lacquer tree (*Rhus vernicifera* STOKES) has been used as an excellent preservative surface coating material for wood, porcelain, and metallic wares in Asian countries. Contact with poison ivy plant and related plant species including laquer tree causes irritation, inflammation, and blistering of the skin. For this reason, there has been great interested in determining the chemical structure of allergenic principles. After many investigations on the active principles that induce allergy, Majima reported a mixture of olefinic catechols having an n-$C_{15}$ alkyl side chain, commonly referred to as urushiol, were the main allergenic component of sap constitutents of lacquer tree(Majima, 1922). There are many reports on isolation of urushiol derivatives in the poison ivy and related plants(Markiewitz et al., 1965; Hill et al., 1934; Adawadkar et al., 1983).

It is interesting to see the old wares coated with lacquer tree sap are well preserved, and this suggest that the sap might contain the strong antioxidants. The mechanism of the oxidative polymerization and cross linking of Japanese lacquer has been studied(Tyman, 1979). However, there is no report on the antioxidative components in the sap of lacquer tree. Therefore, we tried to isolate the components with antioxidative activities in the sap of the Korean lacquer tree (*R. vernicifera*) by a bioassay guided fractionation. As a result, four active components isolated were found to be olefinic catechols. In this paper, we described the isolation of the active compounds and their antioxidative activities.

## MATERIALS AND METHODS

### General

$^1$H-NMR spectra were obtained on a Varian UNITY-300 spectrometer. Mass spectra were recorded with a HEWLETT PACKARD MS-Engine 5989A instrument. IR and UV were measured with a Laser Precision Analytical RFX-65 FT-IR and a Shimadzu UV-260 spectrophotometer, respectively.

### Plant materials

The sap of lacquer tree(*Rhus vernicifera*) was obtained from Wonju, Kwangwon Province, Korea, where is a main production area of Korean lacqure tree, in October 1995.

### Extraction and isolation of active compounds

The sap (37 *ml*) of lacquer tree was diluted to a volume of 1 *l* by the addition of distilled water and extracted with 1 *l* of n-hexane twice. The combined hexane extracts were concentrated under reduced pressure to afford a brownish oil (24.43g). The aqueous layer was further extracted with 1 *l* of ethyl acetate and butanol. The hexane extract (3.64g) showing a free radical scavenging activity was subjected to silica gel column chromatography (5 cm x 70 cm, Merck 7734) 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 (1.6 x 100 cm, YMC GEL ODS-A) with a gradient of methanol and water to give four compounds (1, 1,138.0mg; 2, 158.8mg; 3, 13.1mg; 4, 19.3mg).

### DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was measured according to Xiong et al. One *ml* of 0.15 mM DPPH in ethanol was added to sample solution containing 4 *ml* of methanol and allowed to react for 30 min at room temperature, then the optical density was measured at 517 nm. For the blank, ethanol was used instead of DPPH solution, and for control, methanol was used instead of the sample solution. The $IC_{50}$ values were caculated from the regression lines where the abscissa represented the concentration of tested compound and the ordinate the average percent reduction of DPPH radical from three separate tests.

### Inhibitory activity on lipid peroxidation in rat liver microsomes

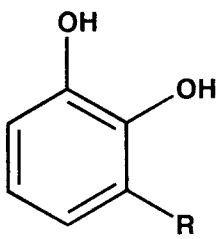
The inhibitory activity on lipid peroxidation in rat liver microsomes was measured by the thiobarbituric acid (TBA) method(Kim et al., 1996). Rat liver microsomes prepared according to the method of Ohkawa et al.(Ohkawa et al., 1979) with some modification were suspended in 100 mM Tris-HCl buffer (pH 7.4). Reaction was initiated by the addition of 100 $\mu$M $FeSO_4 \cdot H_2O$. After 30 min at 37℃ under reciprocal agitation, the reaction was stopped by the addition of 3 M trichloroacetic acid in 2.5 N HCl. Lipid peroxidation was assessed by measuring TBA reactive products. Percent inhibition was calculated as follows: (1-(T-B)/(C-B)) x 100(%), in which T, C, and B are absorbance values at 530 nm of the compound treatment, the control (peroxidation without compound) and the 0 time control (no peroxidation), respectively. Reagents including DPPH were purchased from Sigma.

## RESULTS AND DISCUSSIONS

The sap of Korean laquer tree (*Rhus vernicifera* STOKES) was partitioned between n-hexane and water. The hexane-soluble fraction with a free radical scavenging activity was subjected to silica and ODS gel column chromatography. The antioxidative compounds were identified as four olefinic catechols such as 3-[8' (Z), 11' (Z), 14' -pentadecatrienyl]catechol (1), 3-[8' (Z), 11' (Z)-pentadecadienyl]catechol (2), 3-[8' (Z)-pentadecenyl]catechol (3), and 3-pentadecylcatechol (4) by the comparison of their

$^1$H-NMR , EI-MS, IR and UV with data reported in the literatures (Fig. 1)(Markiewitz et al., 1965; Du et al., 1984; Yamauchi et al., 1982; ElSohly et al., 1982). The four components occupied much more than 25% of the sap. The major component was 1 (over 21% in the sap), followed by 2 (over 3% in the sap). The exact quantity of these components in the tissues of Korean lacquer tree remains to be studied.

Most components in the sap (ca. 68.5%) were extracted by n-hexane, which showed a high scavenging




**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 structures of four urushiol derivatives isolated from the sap of Korean lacquer tree *(Rhus vernicifera)*.

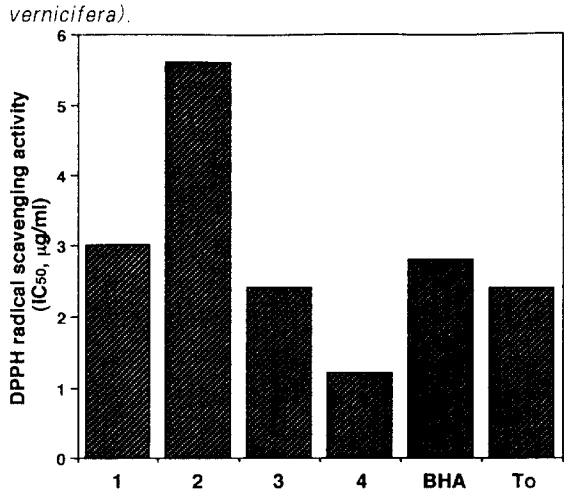



Fig. 2. DPPH radical scavenging activity of four urushiol derivatives isolated from the sap of Korean lacquer tree *(Rhus vernicifera)*. Values were expressed as mean ±S.D. of three replicates. BHA and TO represent 3-*tert*-butyl-4-hydroxyanisole and α-tocopherol, respectively.

activity on DPPH radical ($IC_{50}$: 1.4 μg/ml). Four urushiol derivatives isolated from hexane extracts showed high scavenging activities on DPPH radical (Fig. 2). Four compounds showed slightly different activities depending on the number of double bonds in side chain. Particularly, 4 showed the highest activity ($IC_{50}$: 1.2 μg/ml) which was about two times higher than those of a synthetic antioxidant, 3-tert-butyl-4-hydroxyanisole (BHA) and α-tocopherol.

The four compounds isolated from the sap showed strong inhibitory activities on the lipid peroxidation in rat liver microsomes (Fig. 3). The activities ($IC_{50}$) of four urushiol derivatives showed a similar level ranging from 2.1 to 3.5 μg $ml^{-1}$, which is about two times higher than that of an authentic α-tocopherol.

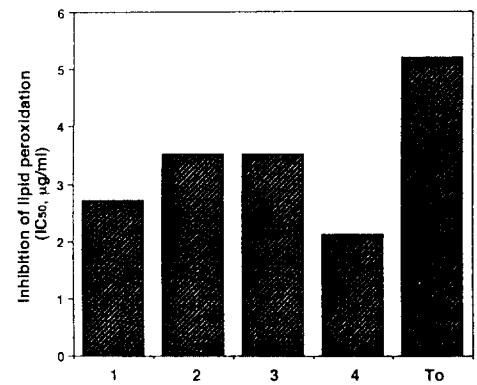



Fig. 3. Inhibitory activity on lipid peroxidation in rat liver microsomes of four urushiol derivatives isolated from the sap of Korean lacquer tree *(Rhus vernicifera)*. Values were expressed as mean±S.D. of three replicates. TO represents α-tocopherol.

Four olefinic catechols isolated in this study are well known allergenic chemicals, commonly referred to as urushiol, found in poison ivy plant and related plants(Markiewitz et al., 1965; Hill et al., 1934; Adawadkar et al., 1983). Jung et al analyzed the components of Korean lacquer tree, but there were no reports on the bioactive components in the same material(Jung et al., 1990). To our knowledge, this is the first report on the antioxidative characteristics of the four urushiol derivatives. The sap of oriental lacquer tree (*Rhus vernicifera*) has been used as an excellent

preservative surface coating material for wood, porcelain, and metallic wares in Asian countries for thousands of years(Onishi, 1995; Tyman, 1979). In fact, the oldest woody relics were coated with lacquer tree sap. From our results, urushiol derivatives may be contributed to chemical defensive characters of wares coated with lacquer tree sap against oxidative stress. 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 lacquer tree have 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 activities of urushiol derivatives, they have diverse biological activities including antifungal and anticancer activities (Detailed data will be published in elsewhere). Thus, urushiol derivatives are good sources for industrial applications such as coating materials and medicine. The *in vivo* test of active compounds using mouse is under investigation.

## ACKNOWLEDGEMENTS

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