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Antifungal Activity of Urushiol Components in the Sap of Korean Lacquer Tree (*Rhus vernicifera* Stokes)

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ABSTRACT

Four urushiol components isolated from the sap of Korean lacquer tree (Rhus vernicifera Stokes) showed a strong antifungal activity, but they have no or low activity against the bacteria and yeasts. Among them, 3-pentadecylcatechol marked the highest activity on the spore germination of Cladosporium herbarum (MIC: 4μg/ml).

Key word: *Rhus vernicifera*, lacquer tree, urushiol, antifungal activity

INTRODUCTION

Korean lacquer is made from the sap of the Korean lacquer tree (*Rhus vernicifera* STOKES), a member of the Anacardiacae plant family. The sap of oriental lacquer tree has been used as an excellent preservative surface coating material for wood, porcelain, and metallic wares in Asian countries for thousands of years (Kumanotani, 1983; Tyman, 1979). The 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, they were determined to be mixture of olefinic catechols with an n-C_{15} or n-C_{17} alkyl side chain, commonly referred to as urushiol(Majima, 1922). After that report, there were many reports on isolation of urushiol components in the poison ivy and related plants (Markiewitz et al., 1965; Hill et al., 1934; Adawadkar et al., 1983).

It is interesting to see that the old wares coated with lacquer tree sap is well preserved, suggesting that the sap might contain the strong antistress-related chemicals such as antimicrobial and antioxidative components. However, there is no report on the antistress-related chemicals in the sap of lacquer tree. In the previous paper, we isolated four olefinic catechols with strong antioxidative activities from the sap of Korean lacquer tree (Kim et al., 1997). Interestingly, the antimicrobial components in the sap of Korean lacquer tree were the same as four antioxidative olefinic catechols. In this paper, we describe the antimicrobial activities of four olefinic catechols.

The sap of Korean laquer tree was partitioned between n-hexane and water. The hexane-soluble fraction was subjected to silica and ODS gel column chromatography by a bioassay guided fractionation. Finally, the active components were identified as four olefinic catechols such as 3-(8' Z, 11' E, 13' Z-pentadecatrienyl) catechol (1), 3-(8' Z, 11' Z-pentadecadienyl]catecho) (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 (Markiewitz et al., 1965; Du et al., 1984; Yamauchi et al., 1982; ElSohly et al., 1982).

The antimicrobial activities of four olefinic catechols isolated from the sap of Korean lacquer tree and n-hexane extracts are summarized in Table 1. The purified compounds and n-hexane extracts marked a strong inhibition

activity on the spore germination of *Cladosporium herbarum*, whereas they showed very low antibacterial activites on *Bacillus subtilis* and *Escherichia coli*. The n-hexane extracts showed a relatively high antifungal activity (MIC: 16μg /ml). Purified compounds showed slightly different activities depending on the number of double bonds in side chain. Among them, 3-pentadecylcatechol (4) showed the highest activity (MIC: 4 μg/ml). However, these components have no or low activity against the bacteria (Table 1.) as well as yeasts (data not shown here).

found at the Kaboto ruins near the mouth of the Yangtze Kiang, China was well kept its clear color and shape for 7000 years (Onishi, 1995). Raw lacquer used in this study as a starting material is a viscous material which is composed of about 60% urushiol and its oligomer. When raw lacquer dries, urushiol is polymerized to form a hard film (Kawai et al., 1992). Thus, the changes of biological activities during polymerization of urushiol remain to be studied. The polymerization of naturally occurring phenolics showed a variety of bioactive products, whereas the substrate

Table 1. Antimicrobial activities of hexane extract and four urushiol components from the sap of Korean lacqure tree(*Rhus vernicifera*).

Test organism	MIC*(μg/ml)				
	hexane layer**	1	2	3	4
Bacillus subtilisa	500	500	1000	500	250
Escherichia colia	1000	1000	500	>1000	500
Staphylococcus aureusa	>1000	1000	1000	1000	1000
Asperigillus awamorib	500	250	500	500	250
Cladosporium herbarumb	16	8	16	8	4
Penicillium oxalicumb	500	250	500	250	250

aDilution method; bSpore germination test

*The MIC values against bacteria and fungi were determined by the serial 2-fold dilution method. The growth of the bacteria was evaluated by the degree of turbidity of the culture with the naked eye, and the sporegermination of fungi was examined under a microscope.

**n-Hexane extract of the curde sap of lacqure tree.

1:3-[8′ Z, 11′ Z, 14′ Z-pentadecatrienyl]catechol
2:3-[8′ Z, 11′ Z-pentadecadienyl]catechol
3:3-[8′ Z-pentadecenyl]catechol
4:3-pentadecylcatechol

Four olefinic catechols showing antifungal and antioxidative activities are well known allergenic 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). To our knowledge, this is the first report on the antimicrobial characteristics of urushiol components. The sap of oriental lacquer tree (*R. vernicifera*) has been used as an excellent preservative surface coating material for wood, porcelain, and metallic wares in Asian countries for thousands of years (Kumanotani, 1983; Tyman, 1979). In fact, the oldest woody relics were coated with lacquer tree sap: the lacquer ware has no or very low biological activity (Kobayashi et al., 1994). From our results, urushiol components may be contributed to chemical defensive characters of wares coated with lacquer tree sap against fungal infestation and oxidative stress. Urushiol components could be developed for useful antifungal reagents as well as antioxidants for various applications.

EXPERIMENTAL

Plant materials

The sap of lacquer tree (*Rhus vernicifera*) was

obtained from Wonju, Kwangwon Province, Korea, which is a main production area of Korean lacqure tree, in October 1995. Taxonomic identification was verified by Mr. Kihun Song, Chollipo Arboretum, Chungnam, Korea and voucher specimens are deposited in Korea Research Institute of Bioscience and Biotechnology, KIST.

Extraction and isolation of active components

The sap of lacquer tree (37 ml) was filled up to a volume of 1000 ml by the addition of distilled water and extracted twice with 1000 ml of n-hexane. The combined n-hexane extracts were concentrated under reduced pressure to afford a brownish oil (24.43 g). The 3.64 g of hexane extract was succesively purified by using silica gel (two times) and ODS (one time) gel column chromatographies to give four olefinic catechols, 3-(8' Z, 11' E, 13' Z-pentadecatrienyl)catechol (1, 19.3mg), 3-(8' Z, 11' Z-pentadecadienyl)catechol (2, 13.1 mg), 3-(8' Z-pentadecenyl)catechol (3, 158.8 mg), and 3-pentadecylcatechol (4, 1,138.0 mg). The detail purification steps were described in the previous paper (Kim et al., 1997).

Antimicrobial test.

The MIC values against fungi and bacteria were determined by the serial 2-fold dilution method (Kobayashi et al., 1994). Fungi was incubated on the slant culture using a potato-malt extract-sucrose agar medium at 27℃ for 7 days to form a well-expanded fungal mat with spores. These spores were suspended in 50 ml of a liquid medium containing 0.2% glucose, 0.1% yeast extract, 0.37% $Na_2HPO_4 \cdot 12H_2O$ and 0.1% citric acid monohydrate. Spore-suspension cultures containing the test sample were placed in the wells of a 96-well microplate at 27℃ for 24 h. The spore germination was examined under a microscope. Bacteria were pre-cultured in 10 ml of a nutrient-broth medium for 12 h at 27℃ on a shaker, and then diluted 100-fold with the same medium. The growth of the bacteria was evaluated by the degree of turbidity of the culture with the naked eye.

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