

Inhibition of Tyrosinase and Lipoyxygenase Activities by Resveratrol and Its Derivatives from Seeds of *Paeonia lactiflora*

– Research Note –

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Abstract

Previously, a methanol extract from seeds of *Paeonia lactiflora* was shown to have a potent inhibitory activities against tyrosinase and soybean lipoyxygenase (SLO). Seven stilbenes, *trans*-resveratrol-4-O- β -D-glucoside, *trans*-resveratrol, *trans*- ϵ -viniferin, *cis*- ϵ -viniferin, gnetin H, suffruticosol A and B were isolated from the seeds as active principles for inhibition of the enzymatic activity. Among them, the resveratrol trimer, gnetin H exhibited the most potent inhibitory activities against tyrosinase and SLO, respectively. Additionally, the resveratrol dimers, *trans*- ϵ -viniferin and *cis*- ϵ -viniferin exhibited significant inhibitory activity against the two oxidative enzymes. Meanwhile, three other stilbene derivatives, such as *trans*-resveratrol, suffruticosol A and suffruticosol B had also weak inhibition activity. The least inhibitory activity was observed in *trans*-resveratrol-4-O- β -D-glucoside. These results suggest that resveratrol dimers and trimer in the seeds of *Paeonia lactiflora* are potentially useful therapeutic agents against pathological disorders such as hyperpigmentation and inflammation.

Key words: *Paeonia lactiflora* Pall., Paeoniaceae, tyrosinase, soybean lipoyxygenase, stilbene derivatives

INTRODUCTION

Tyrosinase (monophenol, dihydroxyphenylalanine: oxygen oxidoreductase EC 1.14.18.1) catalyzes the oxidation of L-tyrosine to L-DOPA and L-DOPA to dopaquinone. These reactions are the initial steps of melanin biosynthesis, a determinant of skin colour which is involved in local human hyperpigmentation diseases, such as melasma, ephelide, and lentigo (1,2). Therefore, tyrosinase inhibitors are widely used as skin-whitening agents in cosmetics.

Lipoyxygenase (linoleate: oxidoreductase, EC 1.13.11.12) catalyzes the dioxygenation of polyunsaturated fatty acids possessing a 1,4-*cis,cis*-pentadiene moiety to yield 1,3-*cis*, *trans*-diene 5-hydroperoxides. In particular, a soybean lipoyxygenase (SLO) is mainly responsible for the production of undesirable off-flavors during oil processing and is known to have similar mechanistic action to a 5-lipoyxygenase (5-LO) in animals (3,4). 5-LO, which is a key enzyme involved in arachidonic acid metabolism, catalyzes the oxygenation of arachidonic acid to 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE) and its subsequent dehydration to form several leukotrienes (LTs). LTs are well-known to play important physiological roles in several pathological disorders, including inflammation and allergy (5,6). Therefore, the specific SLO inhibitors have potential as therapeutic drugs for the prevention

and/or treatment of these diseases.

The root of *Paeonia lactiflora* Pallas (Paeoniaceae) is widely employed as a sedative and antispasmodic agents in traditional Chinese medicine. Many extensive studies on the chemistry and pharmacology of the roots have been performed (7-9). However, few phytochemical studies of the seed are available. Recently, we have isolated resveratrol and its derivatives possessing anticarcinogenic, antimutagenic and antioxidative activities from the seed (10,11). Additionally, the seeds were found to have strong inhibitory activities against mushroom tyrosinase and soybean lipoyxygenase, and resveratrol and its dimer, viniferin were acted as active principles for inhibition of the enzymes (12,13). However, systematic studies on the inhibition of tyrosinase and lipoyxygenase by resveratrol and its derivatives have not been reported. In this study, seven stilbenes, including resveratrol and its glucoside and oligomers, were isolated from the seeds of *Paeonia lactiflora* and their inhibitory activities against mushroom tyrosinase and SLO were determined.

MATERIALS AND METHODS

Materials

The seeds of *Paeonia lactiflora* Pallas were directly harvested in mid August in the herb garden of Uisong

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Medicinal Plant Experiment Station, Gyeongbuk, Korea. A voucher specimen has been retained in the Department of Food Science and Nutrition, Catholic University of Daegu. Mushroom tyrosinase, L-dihydroxyphenylalanine (L-DOPA), soybean lipoxygenase (type V), linolenic acid, trifluoroacetic acid (TFA), L-ascorbic acid, and nordihydroguaiaretic acid (NDGA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and other chemicals used in this experiment were of analytical grade.

Extraction and fractionation

Ground seeds (300 g) of *Paeonia lactiflora* were extracted continuously with MeOH at room temperature, filtered, and then evaporated under a reduced pressure. The methanol extract (37.5 g) was solubilized in 80% aqueous MeOH (500 mL) and then defatted twice with *n*-hexane (1 L). The concentrated 80% aqueous MeOH extract (25.5 g) was redissolved in 60% aqueous MeOH and filtered. The filtrate was diluted with D-H₂O and subjected onto Diaion HP-20 (Mitsubishi Chem. Co., Japan) column (5.5 × 60 cm). The column was eluted successively with 20% aqueous MeOH (4 L), 40% MeOH (5 L), 60% MeOH (12 L), 80% MeOH (5 L) and 100% MeOH (3 L), and then concentrated to attain 20% MeOH fr. (0.17 g), 40% MeOH fr. (0.8 g), 60% MeOH fr. (2.92 g), 80% MeOH fr. (7.24 g) and 100% MeOH fr. (0.32 g). Among the five fractions isolated, the 40% MeOH fr. (0.8 g) was chromatographed repeatedly on a Sephadex LH-20 (Pharmacia Fine Chemicals, Sweden) column (2.5 × 50 cm) with 80% aqueous MeOH to separate compound 1 (330 mg). The 60% MeOH fr. (2.92 g) and 80% MeOH fr. (7.24 g) were combined and chromatographed on silica gel (70–230 mesh, Merck, Germany) column (5.5 × 50 cm) with CHCl₃-MeOH (4:1, v/v) as an eluent to afford five fractions; Fr. 1 (0.062 g), Fr. 2 (0.19 g), Fr. 3 (0.41 g), Fr. 4 (1.29 g) and Fr. 5 (3.78 g). Fr. 2 was chromatographed on a Sephadex LH-20 column (2.5 × 80 cm) with MeOH to separate compound 2 (51 mg). Fr. 3 was further purified by preparative HPLC (Waters Delta Prep 4000, USA) using a Waters RCM Prep Nova-pack HR C18 column (25 × 100 mm). The solvent was eluted isocratically with 0.1% trifluoroacetic acid (TFA) in 45% MeOH at a flow rate of 5 mL/min, monitored at UV_{320nm} to isolate compound 3 (35 mg, Rt. 15.5 min) and compound 4 (173 mg, Rt. 23.6 min). Fr. 4 was also chromatographed on a Sephadex LH-20 column (2.5 × 80 cm) with MeOH to separate compound 5 (0.34 g). Finally, Fr. 5 was subjected to the same purification procedure on Sephadex LH-20 column with MeOH, which afforded pure compound 6 (0.83 g) and compound 7 (1.44 g). A schematic procedure for the isolation and purification of the seven stilbenes from seeds of *Paeonia lactiflora* is shown in Fig. 1. The

seven compounds 1–7 (Fig. 2) had already been characterized as *trans*-resveratrol-4-*O*-β-D-glucoside, *trans*-resveratrol, *trans*-ε-viniferin, *cis*-ε-viniferin, gnetin H, suffruticosol A and B, respectively, in previous reports (10,11).

Assay of tyrosinase activity

DOPA oxidase activity of mushroom tyrosinase was spectrophotometrically determined according to the method of Shin et al. (14). The reaction mixture containing L-DOPA (25 mM), 10 mM sodium phosphate buffer (pH 6.8) and samples (in DMSO) at various concentrations were added to 96-well microplate (TPP, Switzerland) and mixed with mushroom tyrosinase. After incubation at 37 °C for 20 min, the amount of dopachrome produced in the reaction mixture was determined from the optical density at 492 nm (OD₄₉₂) by using a microplate reader (Bio-Rad model 550, Japan). The percentage tyrosinase inhibition was calculated as follows: $[1 - (\text{sample OD}_{492} / \text{control OD}_{492})] \times 100$.

Assay of soybean lipoxygenase (SLO) activity

SLO activity was spectrophotometrically determined by a slightly modified procedure of Block et al. (15) as described previously (13). The reaction mixture containing Tris buffer (pH 8.5), samples at various concentrations, and SLO was incubated at 25 °C for 1 min, after which lipid peroxidation was initiated by the addition of linolenic acid as the substrate. The change of absorbance at 234 nm was recorded as a function of time on a spectrophotometer. The rates were measured from the initial slopes of the linear portions of the curves. A sample containing all of the reagents except for the enzyme and sample solutions was used as a blank and control sample. A curve plotting concentration against percentage inhibition of the two enzymes was used to calculate half maximal inhibition concentration (IC₅₀).

Statistical analysis

Data are expressed as mean ± standard deviation (SD) of three replicates. Statistical analysis was performed using Duncan's Multiple range test at $p < 0.05$ (16).

RESULTS AND DISCUSSION

Recently, resveratrol and its derivatives have received much attention as biologically active compounds due to their variety of physiological and pharmacological actions. Previously, we found that *Paeonia lactiflora* seed, currently an unused plant seed, contained considerably large amounts of resveratrol and its glucosides and oligomers (10,11), suggesting that the seeds have potential as sources of important crude drugs or functional ingredients.

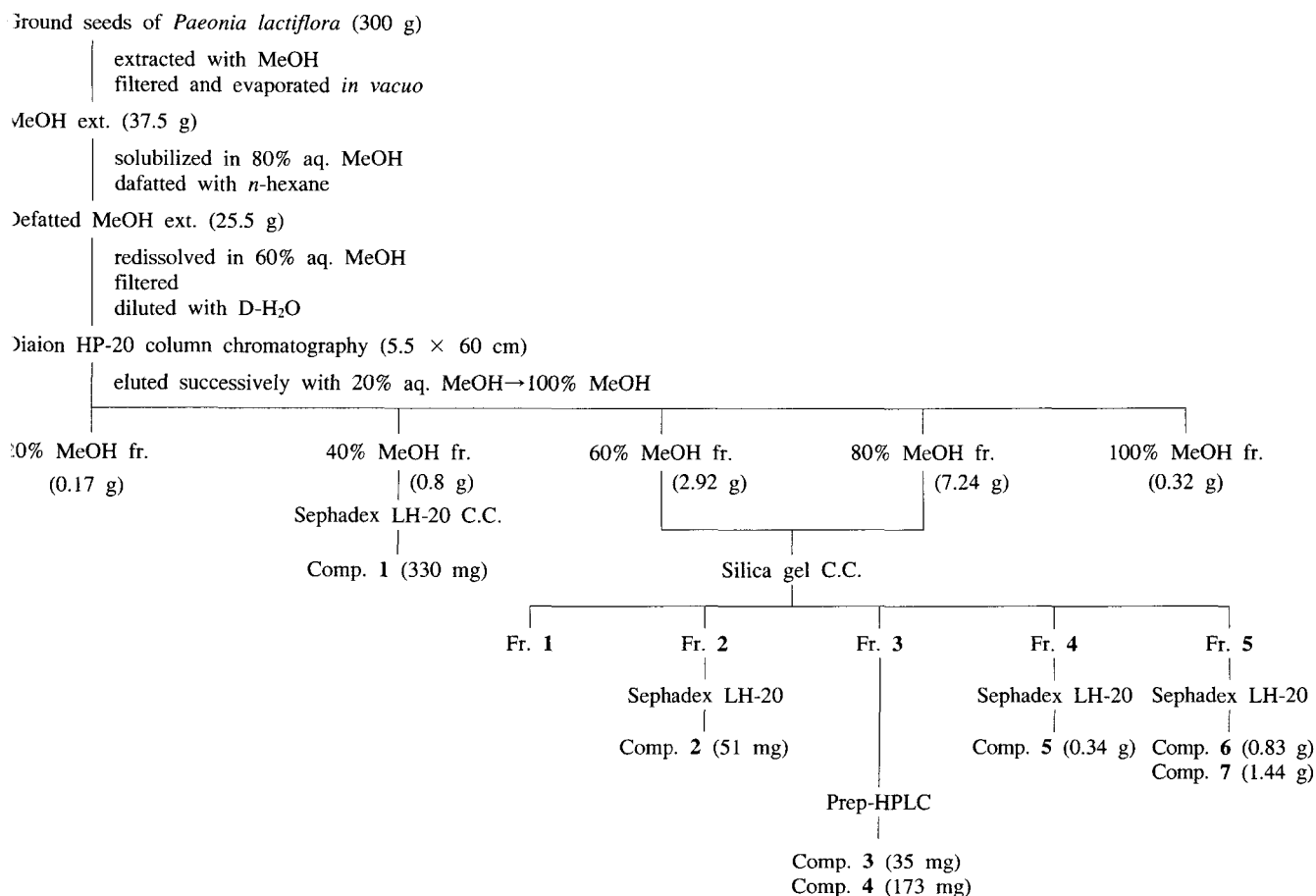


Fig. 1. Schematic procedure for the isolation and purification of seven stilbenes from seeds of *Paeonia lactiflora*.

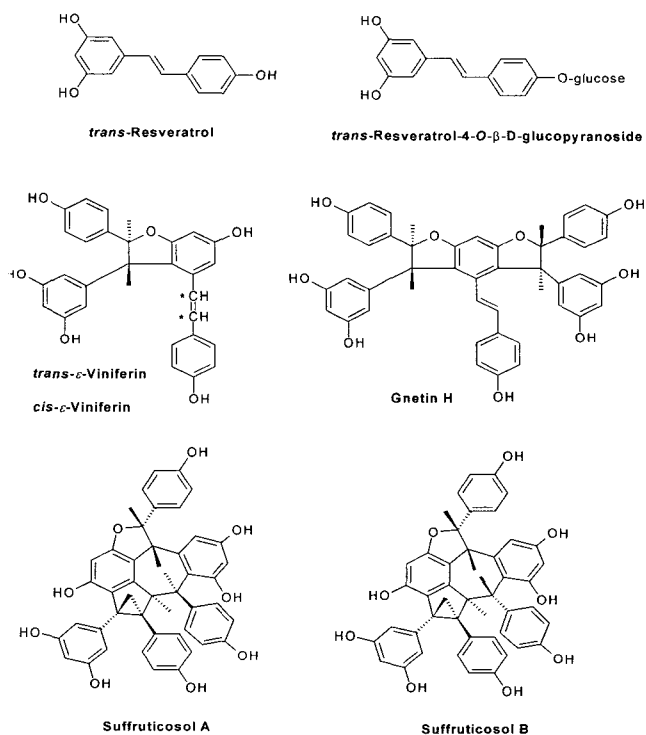


Fig. 2. Chemical structures of seven stilbenes isolated from seeds of *Paeonia lactiflora*.

To screen the seeds for potential functional materials in foods, cosmetic and medicines, we performed successively large scale isolation of stilbenes derivatives from the seed using an ion exchange resin, Diaion HP-20, instead of solvent fractionation as reported previously (10,11).

Inhibitory activities of seven stilbenes 1-7 isolated from the seed against mushroom tyrosinase and soybean lipoxygenase (SLO) are shown in Table 1. Among them, the resveratrol trimer, gnetin H exhibited potent tyrosinase ($IC_{50}=36.7 \mu M$) and lipoxygenase ($IC_{50}=1.5 \mu M$) inhibitory activities. Tyrosinase inhibitory activity of gnetin H was stronger than that of a L-ascorbic acid ($IC_{50}=61.4 \mu M$), although its activity toward SLO was lower than that of the well-known lipoxygenase inhibitor of nordihydroguaiaretic acid (NDGA) ($IC_{50}=0.8 \mu M$). In addition, resveratrol dimer, *trans*- ϵ -viniferin and *cis*- ϵ -viniferin also showed significant inhibition activity against the two oxidative enzymes, while *trans*-resveratrol and its trimers, suffruticosol A and suffruticosol B showed weak inhibitory activity, and only against SLO. However, resveratrol glucoside was a relatively less effective inhibitor of the enzymes, which was ascribed to a steric hindrance by the bulky

Table 1. Inhibitory effects of resveratrol and its derivatives isolated from *Paeonia lactiflora* seeds on a tyrosinase and soybean lipoxygenase (SLO) activities

Compound	Tyrosinase inhibition (IC ₅₀ , μ M) ¹⁾	SLO inhibition (IC ₅₀ , μ M) ¹⁾
<i>trans</i> -Resveratrol	146.3 \pm 2.7 ^{b4)}	24.5 \pm 1.5 ^b
<i>trans</i> -Resveratrol-4- <i>O</i> - β -D-glucoside	650.6 \pm 3.8 ^a	86.4 \pm 2.7 ^a
<i>trans</i> - ϵ -Viniferin	51.5 \pm 1.7 ^c	2.7 \pm 0.4 ^d
<i>cis</i> - ϵ -Viniferin	56.3 \pm 1.4 ^c	2.0 \pm 0.3 ^d
Gnetin H	36.7 \pm 1.2 ^d	1.5 \pm 0.2 ^d
Suffruticosol A	152.5 \pm 2.6 ^b	7.2 \pm 0.4 ^c
Suffruticosol B	153.6 \pm 2.5 ^b	3.5 \pm 0.5 ^c
NDGA ²⁾		0.8 \pm 0.1 ^c
L-Ascorbic acid ³⁾	61.4 \pm 0.9 ^c	

¹⁾IC₅₀ values represent the concentration of sample causing 50% inhibition of tyrosinase and SLO activities.

²⁾NDGA, nordihydroguaiaretic acid, and ³⁾L-ascorbic acid were used as positive references.

⁴⁾Values with different superscripts within the same column are significantly different at $p < 0.05$.

glucosyl moiety (14).

Resveratrol and its derivatives, naturally occurring phytoalexins (17), are known to act as inhibitor of enzymes, such as cyclooxygenase, lipoxygenase, tyrosinase, protein-tyrosine kinase (PTK) and protein kinase C (PKC), which are closely related to human disease processes, including cancer, inflammation, hyperpigmentation, and reperfusion injury (18-20). Our results suggest that resveratrol dimer and trimer, such as viniferin and gnetin H in seeds of *Paeonia lactiflora* may be potentially useful as therapeutic agents for the treatment of hyperpigmentation and inflammation. Further studies on the inhibitory activity of seven stilbene derivatives, from the seeds, on B-16 mouse melanoma cells and 5-LO derived from peritoneal polymorphonuclear leukocytes (PMNL) of rats, as well as structure-activity relationships, are currently under investigation in our laboratory.

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(Received October 16, 2002; Accepted December 7, 2002)