Isolation of Antioxidative Phenolic Glucosides from Lemon Juice and Their Suppressive Effect on the Expression of Blood Adhesion Molecules

Yoshiaki MIYAKE,¹,† Mika MOCHIZUKI,¹ Miki OKADA,² Masanori HIRAMITSU,² Yasujiro MORIMITSU,³ and Toshihiko OSAWA⁴

¹Faculty of Human Wellness, Tokaigakuen University, Nagoya 468-8514, Japan
²Pokka Corporation Ltd., Kitanagoya 481-8515, Japan
³Laboratory of Food Chemistry, Department of Nutrition & Food Science, Ochanomizu University, Tokyo 112-8610, Japan
⁴Laboratory of Food and Biodynamics, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

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Phenolic glucosides having radical scavenging activity were examined from the fraction eluted with 20% methanol on Amberlite XAD-2 resin applied to lemon (Citrus limon) juice by using reversed phase chromatography. Four phenolic glucosides were identified as 1-feruloyl-C₁₂-D-glucopyranoside, 1-sinapoyl-C₁₂-D-glucopyranoside, 6,8-di-C-glucosylapigenin and 6,8-di-C-glucosyldiosmetin by ¹H-NMR, ¹³C-NMR, and MS analyses. They exhibited radical scavenging activity for 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide, although the activity was low in comparison with eriocitrin, a potent antioxidant in lemon fruit, and the eriodictyol of its aglycone. The phenolic compounds in lemon juice were examined for their suppressive effect on the expression of blood adhesion molecules by measuring the expression of intercellular adhesion molecule-1 (ICAM-1) in human umbilical vein endothelial cells (HUVECs) induced by necrosis factor-α (TNF-α). 6,8-Di-C-glucosylapigenin, apigenin, and diosmetin of the flavones were found to significantly suppress the expression of ICAM-1 at 10 μM (P < 0.05). The phenolic glucosides isolated in this study were contained in comparative abundance in daidai (Citrus aurantium) and niihime (Citrus unshiu × Citrus tachibana) among the sour citrus juices.

Key words: phenolic glucoside; lemon juice; antioxidant; intercellular adhesion molecule-1 (ICAM-1)

Epidemiological studies have indicated that an increased intake of such polyphenolic phytochemicals as flavonoids and phenylpropanoids found in many vegetables and fruits was associated with a decreased risk of cardiovascular disease.¹ It has been suggested that oxidative stress plays an important role in the pathogenesis of cardiovascular diseases, mainly through oxidative modification of low-density lipoprotein (LDL) which initiates vascular inflammation and atherosclerotic lesion formation.² Polyphenolic phytochemicals may therefore have great potential to delay LDL oxidation with their radical scavenging activity and prevent cardiovascular disease.³ Moreover, flavonoids have been shown to be anti-inflammatory agents that inhibit the expression of vascular cell adhesion molecules.⁴ The induction of vascular cell adhesion molecules is a common feature in inflammatory environments and occurs with the early development of atherosclerosis. Cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) have been observed in atherosclerotic lesions and at sites predisposed to lesion formation in coronary atherosclerotic plaques.⁵ The suppressive effect of flavonoids on the expression of cell adhesion molecules in human umbilical vein endothelial cells (HUVECs) induced by tumor necrosis factor-α (TNF-α) has been examined.⁶ Polyphenolic compounds were evaluated in this way for their antioxidative activity and for their suppressive effect on the expression of cell adhesion molecules.⁷,⁸ Lemon (Citrus limon) juice contains such biofunctional components as flavonoids, carotenoids, and ascorbic acid.⁹ Eriocitrin (eriodictyol 7-O-β-rutinoside) and...
Materials and Methods

Materials. A variety of citrus fruits, consisting of kabosu (Citrus sphaerocarpa, produced in Ohita, Japan), lemon (Citrus limon, produced in California), lime (Citrus aurantifolia, produced in Mexico), shiikuwasha (Citrus depressa, produced in Okinawa, Japan), sudachi (Citrus sudachi, produced in Tokushima, Japan), and yuzu (Citrus junos, produced in Miyazaki, Japan) were purchased from a supermarket. Daidai (Citrus aurantium) and niihime (Citrus unshiu × Citrus tachibana) were obtained from Mie Prefectural Science and Technology Promotion Center, Agricultural Research Division, Japan. Eriocitrin was purified from lemon peel.\(^{(17)}\) 6,8-Di-C-glycosylapigenin and 6,8-di-C-glucosylsidosmetin were presented by Dr. A. Sawabe of Department of Agriculture, Kinki University (Nara, Japan). The reagents used in this study were of analytical or HPLC grade (Wako Pure Chemical Industries, Osaka, Japan).

Isolation of the phenolic compounds. Lemon juice (5.0 kg) was obtained by extracting the juice (FMC Technologies, Ehime, Japan) of 130 lemons and centrifuging at 5,000 g to remove the pulp. The lemon juice was put into a reversed-phase column (Φ37 × 500 mm, Amberlite XAD-2 resin, Rohm and Haas Co., Philadelphia, USA). The column was washed with 2-liter of water and eluted with 2-liter of 20% methanol, the eluate then being concentrated under reduced pressure. The concentrated extract was applied to preparative HPLC (LC-8A, Shimadzu Co., Kyoto, Japan) which was carried out in a YMC-ODS column (YMC-Pack ODS-A, Φ20 × 250 mm, S-5 μm, YMC Co., Kyoto, Japan) with UV detection of 330 nm, a mobile solvent of 20% methanol, and a flow rate of 10 ml/min at room temperature. The fractions from each peak separated by this preparative HPLC were checked for their antioxidative activity by a DPPH radical scavenging assay, and the antioxidative fraction was concentrated under reduced pressure. Four compounds (LJ1-4) having antioxidative activity were isolated and purified. LJ1 (53 mg), LJ2 (18 mg), LJ3 (42 mg), and LJ4 (230 mg) were isolated, the purity of LJ1-4 being more than 99% by an HPLC analysis.

DPPH radical scavenging activity. A 96-well microplate was used for the DPPH radical scavenging assay.\(^{(18)}\) The phenolic compounds of LJ 1-4, eriocitrin, hesperidin, and their aglycones (ferulic acid, sinapic acid, apigenin, diosmetin, eriodictyol, and hesperetin) were dissolved in DMSO to prepare samples of 10 mM, before being diluted with methanol to prepare samples of 0.2 mM each. 100 μl of 0.13 mg/ml of DPPH dissolved in ethanol, 90 μl of a 0.1 M Tris-HCl buffer (pH 7.4), and 10 μl of the sample were mixed in the microplate. After a 1-h incubation at room temperature, the absorbance was recorded at 517 nm with a plate reader (Sunrise Rainbow, Wako Pure Chemical Industries, Osaka, Japan). A negative control was run with a solvent sample in place of the test sample. α-Tocopherol as the reference antioxidant was used for a positive control. Each sample and the standard antioxidant were assayed at a final concentration of 20 μM. Each result is expressed as the equivalent per mole of α-tocopherol from the percentage decrease with respect to the negative control value. Each data value is presented as the mean ± SD (N = 4).

\(\text{HPLC analysis.}\) The contents of LJ1-4 in the juice of sour citrus fruits (daidai, kabosu, niihime, lemon, lime, sudachi, shiikuwasha, and yuzu) were determined by HPLC (900 series, Jasco Co., Tokyo, Japan). Four pieces of each fruit were hand-squeezed after cutting the fruit with a knife. The squeezed juice was filtered through gauze and centrifuged at 15,000 g for 15 min to remove the pulp. The supernatant was passed through a 0.45-μm filter (Nihon Millipore, Tokyo, Japan), and the juice was obtained. The contents of LJ1-4 in each juice (N = 4) were determined by HPLC, using a YMC-ODS column (YMC-Pack Φ4.6 × 250 mm, S-5 μm, YMC Co., Kyoto, Japan), UV detection of 330 nm, mobile solvents of methanol and water containing 5% acetic acid, a flow rate of 1 ml/min, and a column temperature of 40 °C. The mobile solvent was changed by the concentration of methanol (0–10 min, isocratic with 10% methanol; 10–20 min, a linear gradient from 10% to 35% methanol; 20–25 min, a linear gradient from 35% to 100% methanol). The retention times of LJ1-4 for standard flavonoids were 10.66 min (LJ1), 12.70 min (LJ2), 18.99 min (LJ3), and 20.93 min (LJ4). The concentrations (μg/g of juice) of LJ1-4 in juice are shown as the mean ± SD (N = 4). A level of less than 1 μg/g of juice is marked by “nd” (not detected).
Superoxide radical scavenging activity. Samples of 0.2 mM were prepared by the same method as that used for the DPPH radical scavenging assay. The superoxide radical scavenging effect of the phenolic compounds was measured with a WST commercial SOD assay kit (Dojindo Molecular Technologies, Gaithersburg, USA), using the 96-well microplate. The microplate with added reagents was incubated at 37 °C for 30 min, and the absorbance was measured at 450 nm with a microplate reader. The negative control was run with the solvent sample, and trolox and α-tocopherol as antioxidants served for the positive control. Each sample and standard antioxidant were assayed at a final concentration of 8.3 μM. The results are expressed as the percentage decrease with respect to the negative control values, each data value being presented as the mean ± SD (N = 4).

Suppressive activity toward the expression of ICAM-1 on HUVECs. The phenolic compounds of LJ1-4, eriocitrin, hesperidin, and their aglycones (ferulic acid, sinapic acid, apigenin, diosmetin, eriodictyol, and hesperetin) were dissolved with DMSO to prepare samples of 10 mM each. HUVECs were cultured on collagen-coated microplates, the cells were treated with TNF-α (10 ng/ml) (Peprotech EC., London, UK) for 6 h, before being fixed with 1% (w/v) paraformaldehyde. The cells were incubated with a mouse anti-human ICAM-1 preserving-free monoclonal antibody (Chemicon International, Temecula, USA) and then treated with the peroxidase-conjugated goat IgG fraction to mouse IgG (whole molecular, MP Biomedicals, Aurora, USA). After adding a TMB (Dojindo Laboratories, USA). After adding a TMB (Dojindo Laboratories, USA) and then treated with the peroxidase-conjugated goat IgG fraction to

Instrumental analysis. UV–vis absorption spectra for a sample dissolved in methanol were recorded with a U-2000 spectrophotometer (Hitachi High-Technologies Co., Tokyo, Japan). 1H-NMR and 13C-NMR spectra for a sample dissolved in d6-dimethyl sulfoxide containing tetramethylsilane (TMS) as the internal standard were obtained with a JNM-AL-400 NMR instrument (400 MHz for 1H and 100 MHz for 13C, Jeol, Tokyo, Japan). FAB-MS and HR-FAB-MS-NEG data for a sample with 1N HCl-glycerol as the mounting matrix were recorded with a JMS-DX-705 MSStation (Jeol, Tokyo, Japan). The LC–MS (Platform series, Nihon Waters, Tokyo, Japan) analysis was done with a reversed-phase column (Dovelosil ODS-HG-5, 4.6F × 250 mm, Nomura Chemical Co., Aichi, Japan), using mobile solvents of acetic acid and methanol (0.01% acetic acid:methanol containing 0.01% acetic acid = 7:3), ESI-negative detection of the ion mode, a flow rate of 0.8 ml/min, and a column temperature of 40 °C.

1-Feruloyl-β-D-glucopyranoside (LJ1), UV (MeOH) λmax: 329, 233, 217. 1H-NMR (CD3OD, 400 MHz) δ: 7.55 (1H, d, J = 16.0 Hz, H-7), 7.20 (1H, d, J = 1.9 Hz, H-2), 7.09 (1H, dd, J = 2.0, 8.4 Hz, H-6), 6.81 (1H, d, J = 8.0 Hz, H-5), 6.40 (1H, d, J = 16.4 Hz, H-8), 5.57 (1H, d, J = 8.0 Hz, H-1′), 3.89 (3H, s, 3-Ome), 3.85 (3H, s, 5-Ome), 3.80 (2H, s, H-2, H-6), 3.4–3.5 (H-2′–H-5′). 13C-NMR (CD3OD, 100 MHz) δ: 167.4 (C-9), 150.7 (C-4), 149.1 (C-3), 148.0 (C-7), 127.4 (C-1), 124.2 (C-6), 116.4 (C-2), 114.6 (C-8), 111.7 (C-5), 95.7 (C-1′), 78.8 (C-5′), 78.0 (C-3′), 74.0 (C-2′), 71.1 (C-4′), 62.3 (C-6′), 56.4 (3-Ome). FAB-MS m/z 357 [M + H]+. HR-FAB-MS (m/z): MS-NEG, [M – H]−, calcd. for C18H20O9, 355,1029; found, 355,1032.

1-Sinapoyl-β-glucopyranoside (LJ2), UV (MeOH) λmax: 330, 237, 216. 1H-NMR (CD3OD, 400 MHz) δ: 7.72 (1H, d, J = 16.0 Hz, H-7), 6.94 (2H, s, H-2, H-6), 6.43 (1H, d, J = 16.0 Hz, H-8), 5.57 (1H, d, J = 8.0 Hz, H-1′), 3.88 (3H, s, 3-Ome), 3.88 (3H, s, 5-Ome), 3.85 (1H, br, d, J = 12.2 Hz, H-6′), 3.70 (1H, dd, J = 4.8, 12.0 Hz, H-6′), 3.4–3.5 (H-2′–H-5′). 13C-NMR (CD3OD, 100 MHz) δ: 167.3 (C-9), 149.3 (2C, C-3, C-5), 148.2 (C-7), 139.7 (C-4), 126.3 (C-1), 115.1 (C-8), 107.0 (2C, C-2, C-6), 95.7 (C-1′), 78.8 (C-5′), 78.0 (C-3′), 74.0 (C-2′), 71.1 (C-4′), 62.3 (C-6′), 56.8 (3-Ome). FAB-MS, m/z 387 [M + H]+. HR-FAB-MS (m/z): MS-NEG, [M – H]−, calcd. for C18H22O10, 385,1135; found, 385,1136.

6,8-Di-C-β-glucosylapigenin (LJ3, vicenin-2). UV (MeOH) λmax: 342, 273, 254s, 243s. LC–MS, 592.7 m/z [M + H]+. The retention time for LJ3 in the HPLC analysis was consistent with the retention time for 6,8-di-C-β-glucosylapigenin.

6,8-C-β-Diglucosydiosmetin (LJ4), UV (MeOH)
Results and Discussion

Isolation and identification of phenolic compounds from lemon juice

Lemon juice has been reported to contain an abundance of flavanone glycosides as eriocitrin (216 μg/g of juice) and hesperidin (197 μg/g of juice). Eriocitrin has been shown to have potent antioxidative activity for the inhibition of lipid autooxidation and a suppressive effect on oxidative stress in experimental animals. Hesperidin is reported to have hypertensive and anti-inflammatory effects, although it exhibits low antioxidative activity in comparison with eriocitrin. The fraction eluted with methanol by reversed-column chromatography of lemon juice was examined for its antioxidative activity by using a linoleic acid oxidation system. The fraction eluted with 20% methanol exhibited antioxidative activity, although this was lower than the activity with 40% methanol which contained eriocitrin and hesperidin. However, phenolic compounds of hydrophilic antioxidants seemed be present in the fraction with 20% methanol. We attempted in this study to isolate the phenolic compounds in the fraction eluted with 20% methanol and to identify them because hydrophilic functional compounds can be expected to be applicable to drinks and water-soluble foods. The HPLC profile for the fraction eluted with 20% methanol by reversed-column chromatography of lemon juice is shown in Fig. 1. Four antioxidative peaks (LJ1-4) were detected by measuring the antioxidative activity for a DPPH radical scavenging system after the 20% methanol-eluted fraction had been fractionated by preparative HPLC. Four compounds (LJ1-4) were isolated as antioxidants and their chemical structures were examined by structural analyses. LJ1 and LJ2 were analyzed by 1H-NMR, 13C-NMR, and FAB-MS, and identified as 1-feruloyl-D-glucopyranoside and 1-sinapoyl-D-glucopyranoside, respectively. The NMR data for LJ1 and LJ2 are consistent with those reported in the literature as isolated from radish sprouts (Kaiware-daikon, Raphanus sativus L.). LJ1 and LJ2 were phenylpropanoid glucosides as shown in Fig. 2, and have also been reported to be present in hot pepper fruit (Capsicum annuum L.), Adonis aleppica (a herb from the Mesoopotamian region), and German Riesling wine. Hydroxycinnamic acid glycoside had been found in the peel of citrus fruits. Phenolic compounds in lemon juice have recently been examined in details by an LC–MS analysis, but there have been no previous definitive reports of the presence of LJ1 and LJ2 as phenylpropanoid glucosides in lemon juice. We were able to identify in this study LJ1 and LJ2 as phenylpropanoid glucosides in lemon juice. As for LJ3 and LJ4, their retention times from the HPLC analysis are consistent with those of the standards of 6,8-C-β-diglucosylapigenin (vicenin 2) and 6,8-C-β-diglucosydiosmetin, respectively. The data on the molecular weights of LJ3 and LJ4 obtained by the LC–MS analysis are also consistent with the standards. As shown in Fig. 2, LJ3 and LJ4 were respectively identified as 6,8-C-β-diglucosylapigenin and 6,8-C-β-diglucosydiosmetin of flavone glucosides. It has been reported that LJ3 and LJ4 were present in bergamot juice (Italian citrus fruit, Citrus bergamia Risso), orange juice, and lemon peel. Phenolic compounds
in lemon juice have recently been analyzed by an LC–MS analysis, and LJ3 and LJ4 were shown to be present in lemon juice.\textsuperscript{15,16} However, other phenolic compounds that were more hydrophilic than LJ3 and LJ4 could be detected by an HPLC analysis, although they could not be identified by the LC–MS analysis.\textsuperscript{15,16} The results for the retention time by HPLC and the UV spectra in the report of Caristi et al.\textsuperscript{15} indicated that the two unidentified phenolic compounds could have been LJ1 and LJ2. Although the phenylpropanoid glycosides in lemon juice have not previously been clearly identified, we were able to confirm their presence in lemon juice in this study.

**Antioxidative activity of the phenolic compounds**

LJ1-4 and their aglycones (ferulic acid, sinapic acid, apigenin, and diosmetin) were examined for their antioxidative effects by the radical scavenging activity of DPPH and superoxide as shown in Figs. 3 and 4, respectively. The flavanone glycosides (erocitrin and hesperidin), which are abundantly contained in lemon juice, and their aglycones (eriodictyol and hesperetin) were also examined in these assays to compare their activity. LJ1-4 were checked by the radical scavenging activity of DPPH and possessed activity when the antioxidative phenolic compounds were isolated from the fraction in reversed-phase chromatography (Fig. 2). The radical scavenging assay of DPPH (Fig. 3) showed the four compounds to exhibit radical scavenging activity, although this activity was lower than that of erocitrin, a potent antioxidant in lemon juice. However, the activity of LJ1-4 and their aglycones was lower than that of erocitrin, which is reportedly a potent antioxidant in lemon juice. It has been suggested that erocitrin and hesperidin were related with the radical scavenging activity because they have an ortho-dihydroxy group at 3' and 4' of the flavonoid B-ring. Eriocitrin and hesperidin have been reported to exhibit potent antioxidative activity, and the results in this study are consistent with such reported data.\textsuperscript{17,28}

**Suppressive effect on the expression of adhesion molecules**

Some phenolic compounds like flavonoids have been reported to have a suppressive effect on the expression of blood adhesion molecules such as ICAM-1, and can be expected to have some effect on the prevention of arteriosclerosis.\textsuperscript{6–8} In this study, LJ1-4, erocitrin, hesperidin, and their aglycones were examined for their suppressive effect on the expression on blood adhesion molecules of ICAM-1 in HUVECs induced by α-TNF, as shown in Fig. 5. LJ3 and hesperidin exhibited a significantly suppressive effect on ICAM-1 expression at 10μM in the assay, in comparison with the positive control ($P < 0.05$). As for the aglycones, sinapic acid as the aglycone of LJ2 exhibited a significant effect at 10μM ($P < 0.05$), and apigenin and diosmetin as the
aglycones of LJ3 and LJ4 exhibited significant effects at 1 μM ($P < 0.05$) and at 10 μM ($P < 0.01$). LJ4 and hesperetin tended to suppress ICAM-1 expression at 10 μM because their $P$ levels with respect to the positive control were 0.052 and 0.092 in the statistical analysis. These results indicate that apigenin and diosmetin had high activity in the assay. The flavone glucosides are thought to have less effect than their aglycones because LJ3 and LJ4 were weaker than apigenin and diosmetin. Dietary flavonoids such as apigenin have been reported to have a suppressive effect on the expression of adhesion molecules.6–8) This effect of the flavonoid has been reported to be related to the main structural requirements of flavone having a 5,7-dihydroxy substitution of the flavonoid's A-ring and a 2,3-double bond and 4-keto group of the C-ring.8) In this study, LJ3, LJ4, apigenin, and diosmetin appeared to exhibit the suppressive effect on ICAM-1 expression because they had those structural requirements. Therefore, the structural requirement of flavonoids for the effect in this study is considered to be consistent with the report of Lotito et al.8) As for the phenolic compounds of LJ1-4 identified in lemon juice in this study, LJ3 may have been a bioactive phenolic compound, related to the prevention of arteriosclerosis, because it was found to have a suppressive effect on the expression of blood adhesion molecules, even although it has low radical scavenging activity.

As for metabolic process of LJ1-4, LJ1 and LJ2 of the phenylpropanoid O-glucosides are speculated to be absorbed for the aglycones by deglucosidation of the intestinal bacteria or small intestinal epithelial cell β-glucosidase and to exist in the plasma as glucuro- and/or sulfo-conjugates of their aglycones. C-Glycosylflavones have been to be metabolized to the flavones of aglycones by human intestinal bacteria.29) LJ3 and LJ4 of the flavone C-glucosides are also speculated to be metabolized for aglycones by deglucosidation of the intestinal bacteria, and to exist in plasma as glucuro- and/or sulfo-conjugates of their aglycones. However, LJ3 and LJ4 may be absorbed poorly and slowly, because the C-glucoside bond of flavone and glucose seems more difficult than the O-glucoside bond.

**Distribution of phenolic compounds in sour citrus fruits**

As shown in Table 1, the distribution of LJ1-4 was examined in the juice of a variety of sour citrus fruit such
as daidai, kabos, niihime, lemon, lime, shiikuwasha, sudachi, and yuzu which form part of the Japanese diet through soft drinks, alcoholic drinks, seasoning, etc. Niihime (Citrus unshiu × Citrus tachibana) is a new hybrid sour citrus fruit produced on the coast of the Kumano Sea, Mie prefecture, in Japan.\(^\text{18)}\) LJ1-4 were found to exist widely in other sour citrus juices in addition to lemon juice. The LJ1 content was high, more than 60 mg/g, in the juice of daidai and niihime; LJ2 was more than 20 mg/g in the juice of daidai and shiikuwasha; LJ3 was more than 120 mg/g in the juice of daidai and niihime; and LJ4 was more than 50 mg/g in the juice of lemons and limes. Daidai and niihime juices were found to contain an abundance of phenylpropanoid glucosides as LJ1-2 and flavone glucosides as LJ3-4.

Four antioxidative phenolic glucosides as phenylpropanoid glucosides (LJ1 and LJ2) and flavone glucosides (LJ3 and LJ4) were isolated and identified in a fraction of lemon juice in this study which have not previously been reported as isolated antioxidants. These four compounds exhibited lower radical scavenging activity than eriocitrin, a potent antioxidant in lemon fruit. However, LJ3 was found to have a significantly suppressive effect on ICAM-1 expression, which is thought to be related to the progression of atherosclerosis, and LJ4 was found to have a lesser but similar effect. Eriocitrin has been reported to be present in lemon juice (216 \(\mu\)g/g of juice).\(^\text{17)}\) As shown in Table 1, the contents (\(\mu\)g/g of juice) of LJ3 and LJ4 in lemon juice were 11.5 and 57.9, respectively. It is speculated that eriocitrin, LJ3, and LJ4 in lemon juice may have a suppressive effect on the progress of atherosclerosis and that eriocitrin may exert the main. LJ3 was abundantly contained in daidai juice (134.2 \(\mu\)g/g of juice) and in niihime juice (124.4 \(\mu\)g/g of juice). Daidai juice has been reported to abundantly contain neoeriocitrin (eriodictyol 7-\(\beta\)-rhamonoglucoside), which has equivalent antioxidative activity to that of eriocitrin, the content being 396 \(\mu\)g/g of juice.\(^\text{18)}\) Daidai juice was suggested to be the most effective juice of sour citrus, because it contains abundantly bioactive compounds having antioxidative activity and a suppressive effect on the expression of blood adhesion molecules. This study found the bioactive compounds of phenolic glucosides in the juices of sour citrus fruits. We think it will be worth investigating biological functional activity and mechanism of phenolic glucosides in sour citrus juices in future work.

![Fig. 4. Superoxide Radical Scavenging Activity of the Phenolic Compounds Isolated from Lemon Juice.](image-url)

Samples were measured with a WST commercial SOD assay kit. The reagents were incubated at 37 °C for 30 min, and the absorbance was measured at 450 nm. Samples and standard antioxidants (trolox and \(\alpha\)-tocopherol) were assayed at a final concentration of 8.3 \(\mu\)M. Each data value is presented as the mean ± SD (\(N = 4\)).

![Phenolic Glucosides Isolated from Lemon Juice 1917](image-url)
Acknowledgment

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