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Lipoxygenase Inhibitory Constituents from Rhubarb

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Phytochemical study on the ethanol extract of rhubarb led to the isolation of fifteen compounds, including five anthraquinones: chrysophanol (**1**), physcion (**2**), emodin (**7**), chrysophanol-8-*O*-β-D-glucopyranoside (**9**) and emodin-8-*O*-β-D-glucopyranoside (**15**), and ten stilbenes: desoxyrhaponticin (**3**), rhaponticin (**4**), resveratrol (**5**), desoxyrhapotigenin (**6**), rhapontigenin (**8**), piceatannol-3'-*O*-β-D-glucopyranoside (**10**), piceid (**11**), ε-viniferin (**12**), ampelopsin B (**13**) and isorhaponticin (**14**). Their structures were identified by comparing the physicochemical data with those of published papers. Among the isolated compounds, stilbene derivatives (**3**-**6**, **8** and **10**-**14**) showed remarkable inhibitory effect on lipoxygenase with IC_{50} values ranging from 6.7 to 74.1 μM. The inhibition kinetics analyzed by Lineweaver-Burk plots found that they were competitive inhibitors with the linoleic acid at the active site of lipoxygenase. In addition, stilbenes exhibited significantly free radical scavenging activity against $ABTS^{\cdot+}$ with trolox equivalent activity capacity (TEAC) values ranging from 1.16 to 4.64. Whereas, anthraquinone derivatives (**1**-**2**, **7**, **9** and **15**) neither inhibited lipoxygenase nor scavenged free radical $ABTS^{\cdot+}$. These results indicated that stilbene derivatives were considerate to be mainly lipoxygenase inhibitor and free radical scavenger constituents of rhubarb.

Key words: Rhubarb, Anthraquinone, Stilbene, Lipoxygenase, ABTS

INTRODUCTION

Lipoxygenases (LOX) is a key enzyme that catalysis the regio- and stereo-specific oxygenation of polysaturated fatty acid containing a 1(Z), 4(Z)-pentadiene system such as linoleic acid and arachidonic acid to their 1-hydroperoxy-1(E), 4(Z)-pentadiene products (Tea *et al*., 2005). Arachidonic acid metabolism through lipoxygenase (LOX) pathways generates various biologically active lipids that play important roles in inflammation (Kubo *et al*., 2002; Nei *et al*., 2002). Thus, the search for novel LOX inhibitors would be beneficial not only to treat inflammation but also to combat various other diseases such as thrombosis, arthritis, bronchial asthma and tumor progression (Aziz *et al*, 2004; Pinto *et al*., 1999)

Recently, huge amounts and diverse species of natural medicines originating from plants and animals have been used all over the world. Among them, rhubarb is an important and well known medicinal origin plant. It has been used in traditional medicine for the treatment of blood stagnation and as well as a purgative agent (Bae, 2001). Rhubarb is the rhizomes of *Rheum undulatum* L., *R. palmatum*., *R. tanguticum* Maxim., *R. officinale* Baill., and *R. coreanum* Nakai, which are distributed in Korea, Japan and China. Previously, stilbene and anthraquinone derivatives are the mainly components with many biological activities such as anti-inflammatory, anti-diabetic, anti-allergic, cytotoxicity, antioxidant (Kim *et al.,* 1999; Hisashi *et al*., 2001; Sang *et al*., 2005) and anti-carcinogenic (Song *et al.,* 2006). Our biological screening the ethanolic extract of the rhubarb showed significant inhibitory activity against the lipoxygenase. This study deals with isolation and characterization of the constituents from the rhubarb, and as well as structural requirements of active constituents for inhibitory effects on lipoxygenase.

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MATERIALS AND METHODS

General experimental

The organic solvents were supplied from DaeJung Chem-

ical and Metals Co. Ltd, Korea. Column chromatographic packing materials: Kieselgel 60 (0.040-0.063) mm (Meck, Germany), Sephadex LH-20, Pre-coated TLC: Kieselgel 60 $F_{254,}$ RP-18 F_{254} (Merck, Germany). Melting point was taken on Electrothermal apparatus. The IR spectra were determined on a Hitachi 270-30 type spectrometer with KBr discs. FAB-MS was taken in MeOH and obtained using a JEOL JMS-DX 300 spectrometer. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker-AM600 FT-NMR unit and chemical shift are expressed as δ values using TMS as an internal standard. Spots were detected by UV light (254 and 366 nm) and spraying 10% H_2SO_4 with heating. Preparative MPLC was carry out on YAMAZEN MPLC system with pump 540; detector Prep UV-10V; ULTRA PACK ODS-S-50A (11 mm $\times$ 300 mm). For the enzyme inhibition assay, all chemicals used and lipoxygenase (1.13.11.12) type I-B was purchased from Sigma-Aldrich Chemical Co., U.S.A..

Plant material

The cultivated Korean rhubarb rhizome was purchased in July 2006 from Yuseong herbal drug market, Daejeon, Korea, and identified by Professor KiHwan Bae. A voucher specimen (CNU-1345) has been deposited in the herbarium of the College of Pharmacy, Chungnam National University, Korea.

Extraction and isolation

The dried and milled rhizomes of rhubarb (4.7 kg) were extracted with 20 L ethanol, three times. The ethanol extract was combined and concentrated to yield a residue (650 g), which was suspended in water and then successively partitioned with hexane, ethyl acetate (EtOAc) and butanol (BuOH) to afford 32.7 g hexane-, 308 g EtOAc-, and 108 g BuOH- soluble fractions, respectively.

The hexane-soluble fraction was subjected to silica gel column chromatography, eluted with hexane-EtOAc (60:1 ~0:1) to give five fractions (H1~H5). The fraction H1 was rechromatographed on silica gel column, hexane-EtOAc (40:1) as eluting solvent to obtain compound **1** (1500 mg). Compound **2** (500 mg) was yielded from the H2 by using a silica gel column chromatography with hexane-EtOAc (30:1). The EtOAc-soluble fraction was diluted with acetone, and then filtrated through filter paper to give precipitated powder (E1, 20.7 g) and acetone fraction (E2, 226.1 g). The E1 was chromatographed on silica gel column, using $CHCl_3$-MeOH (6:1) as eluting solvent to afford compounds **3** (1.2 g) and **4** (1.3 g). The E2 (100 g) was subjected to silica gel column chromatography, and using the mixtures of $CHCl_3$ and MeOH in increasing polarity (80:1 to 0:1) to separate into six fractions (E2.1~E2.6). Compounds **5** (30 mg), **6** (621 mg) and **7** (220 mg) were obtained from the subfraction E2.2 by using ODS column with MeOH-H_2O (1:1). The subfraction E2.3 was rechromatographed on silica gel column with a stepwise gradient of $CHCl_3$ and MeOH (4:1 to 0:1) to give compounds **8** (247 mg) and **9** (613 mg). The subfraction E2.5 was subjected to an ODS column, eluting with MeOH-H_2O (1:2 to 3:1) to afford compounds **10** (175 mg) and **11** (21 mg). The E2.6 was chromatographed on silica gel column with $CHCl_3$-MeOH (5:1 to 0:1) to divide into five fractions (E2.6.1~E2.6.5). Fraction E2.6.3 was further subjected to MPLC [ODS, 250$\times$10 mm id, MeOH-H_2O (35:75)] to furnish compounds **12** (37 mg) and **13** (76 mg). The BuOH-soluble fraction was applied on a Dianion column, eluting with H_2O-MeOH (100:1 to 0:100) to separate into seven fractions (B1~B7). The fraction B2 was futher chromatographed on a silica gel column, using gradient solvent $CHCl_3$ and MeOH in increasing polarity as eluted solvent to obtain compound **10** (1.6 g). The fraction B3 was subjected on a silica gel column chromatography, eluted with $CHCl_3$-MeOH (7:1 to 1:1) to give compound **14** (1.2 g). The fraction B4 was also subjected to silica gel column chromatography and eluting with $CHCl_3$-MeOH (8:1) to obtain compound **15** (40 mg).

Chrysophanol (1)

Yellow powder; mp: 193-194°C; UV λ_{max} (MeOH): 202, 208 and 217 nm; IR (KBr) ν_{max} cm^{-1}: 3000, 1650, 1615, 1560, 1465, and 1200; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.77 (1H, dd, J = 8.0, 1.6 Hz, H-5), 7.64 (1H, t, J = 8.0 Hz, H-6), 7.60 (1H, d, J = 1.6 Hz, H-4), 7.25 (1H, dd, J = 1.6, 8.0 Hz, H-7), 7.05 (1H, brs, H-2), 2.44 (3H, s, 3-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 192.6 (C-9), 182.0 (C-10), 162.8 (C-1), 162.5 (C-8), 149.5 (C-3), 137.1 (C-6), 133.8 (C-4a), 133.4 (C-10a), 124.7 (C-7), 124.5 (C-2), 121.5 (C-4), 120.1 (C-5), 116.0 (C-8a), 113.8 (C-9a), 22.4 (3-CH_3).

Physcion (2)

Yellow powder; mp: 206-207°C; UV λ_{max} (MeOH): 202, 208 and 217 nm; IR (KBr) ν_{max} cm^{-1}: 2980, 1665, 1560, 1480, 1380,1280, 1230 and 1160; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.62 (1H, s, H-4), 7.36 (1H, d, J = 2.4 Hz, H-2), 7.08 (1H, s, H-5), 6.68 (1H, d, J = 2.8 Hz, H-7), 3.94 (3H, s, OCH_3), 2.45 (3H, s, 3-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 191.0 (C-9), 182.2 (C-10), 166.7 (C-6), 165.3 (C-8), 162.7 (C-1), 148.6 (C-3), 135.4 (C-10a), 133.4 (C-4a), 124.7 (C-2), 121.4 (C-4, 113.8 (C-9a), 108.4 (C-8a), 121.4 (C-5), 106.9 (C-7), 56.29 (6-OCH_3), 22.37 (3-CH_3).

Desoxyrhapontincin (3)

White plate; mp: 219-220°C; UV λ_{max} (MeOH): 217, 302 and 320 nm; IR (KBr) ν_{max} cm^{-1}: 3440, 1610, 1580, 1480; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.44 (2H, brd, J = 8.8 Hz, H-2', 6'), 7.07 (1H, d, J = 16.4 Hz, H-α), 6.89 (1H, d, J =

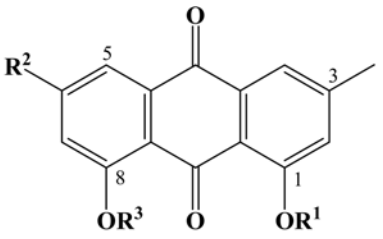
16.4 Hz, H-β), 6.89 (2H, brd, *J* = 8.8 Hz, H-3', 5'), 6.81 (1H, t, *J* = 1.6 Hz, H-2), 6.63 (1H, t, *J* = 1.6 Hz, H-6), 6.47 (1H, t, *J* = 2.0 Hz, H-4), Glu: 4.90 (1H, d, *J* = 7.2 Hz, H-1''), 3.93 (1H, dd, *J* = 2.0, 10.0 Hz, H-6''), 3.79 (3H, s, 4'-OCH_3) 3.47 (1H, m, H-3''), 3.79 (1H, dd, *J* = 1.6, 7.0 Hz, H-5''), 3.48 (1H, m, H-4''); ^{13}C-NMR (100 MHz, CD_3OD) δ: 161.0 (C-4'), 160.6 (C-3), 159.7 (C-5), 141.4 (C-1), 131.5 (C-1'), 129.8 (C-α), 128.9 (C-2', 6'), 127.5 (C-β), 115.2 (C-3', 5'), 108.5 (C-2), 107.2 (C-6), 104.3 (C-4), 102.5 (C-1''), 78.3 (C-2''), 78.4 (C-5''), 75.0 (C-3''), 71.6 (C-4''), 62.7 (C-6''), 55.8 (4'-OCH_3).

Rhaponticin (4)

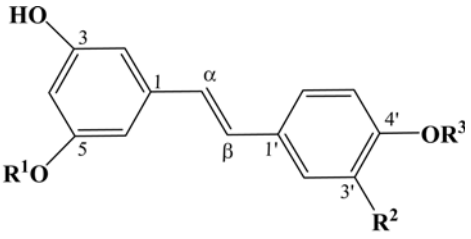
White crystals; mp: 250-251°C; UV λ_{max} (MeOH): 220, 302 and 319 nm; IR ν_{max} (KBr) cm^{-1}: 3440, 1610 and 1510; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.44 (1H, d, *J* = 2.4 Hz, H-2'), 6.97 (1H, d, *J* = 16.4 Hz, H-α), 6.84 (1H, d, *J* = 16.4 Hz, H-β), 6.95 (1H, dd, *J* = 1.6, 6.4 Hz, H-6'), 6.89 (1H, d, *J* = 8.4 Hz, H-5'), 6.79 (1H, brs, H-2), 6.61 (1H, brs, H-6), 6.46 (1H, brs, H-4), 3.86 (4'-OCH_3), Glu: 4.89 (1H, d, *J* = 7.2 Hz, H-1''), 3.93 (2H, dd, *J* = 2.4, 12.0 Hz, H-6''), 3.80 (1H, m, H-3''), 3.71 (1H, m, H-5''), 3.48 (1H, m, H-4''), 3.36 (1H, m, H-2''); ^{13}C-NMR (100 MHz, CD_3OD) δ: 160.6 (C-3), 159.8 (C-5), 149.2 (C-4'), 147.8 (C-3'), 141.3 (C-1), 132.2 (C-1'), 130.1 (C-α), 127.6 (C-β), 120.3 (C-6'), 113.8 (C-2'), 112.8 (C-5'), 108.5 (C-6), 107.2 (C-2), 104.3 (C-4), 102.5 (C-1''), 78.2 (C-5''), 75.1 (C-2''), 71.6 (C-4''), 62.7 (C-6´´), 56.5 (4'-OCH_3).

Resveratrol (5)

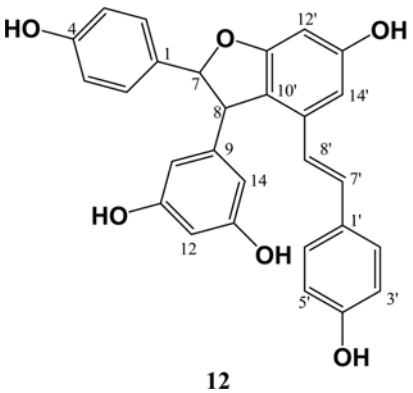
White powder; mp: 257-258°C; UV λ_{max} (MeOH): 220, 302 and 320 nm; IR ν_{max} (KBr): 3340, 1580, 1515 cm^{-1}. ^{1}H-NMR (400 MHz, MeOD) δ: 7.35 (2H, d, *J* = 8.7 Hz, H-2,6), 6.96 (1H, d, *J* = 16.5 Hz, H-α), 6.82 (1H, d, *J*= 16.5 Hz, H-β), 6.77 (2H, d, *J* = 8.7 Hz, H-3,5), 6.46 (2H, d, *J* = 2.1, H-2', 6'), 6.17 (1H, t, H-4'). ^{13}C-NMR (100 MHz, MeOD) δ: 159.7 (C-3', 5'), 158.4 (C-4), 141.5 (C-1'), 130.5 (C-1), 129.6 (C-α), 129.6 (C-2,6), 127.1 (C-β), 116.6 (C-3,5), 105.9 (C-2', 5'), 102.8 (C-4').



	R^1	R^2	R^3
1	H	H	H
2	H	OCH_3	H
7	H	OH	H
9	H	H	Glc
15	H	OH	Glc



	R^1	R^2	R^3
3	Glc	H	CH_3
4	Glc	OH	CH_3
5	H	H	H
6	H	H	CH_3
8	H	OH	CH_3
10	H	Glc	H
11	Glc	H	H
14	Glc	OCH_3	H



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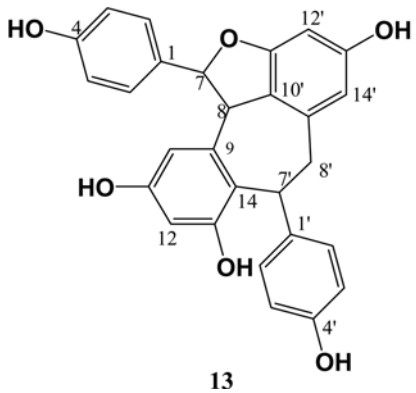

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Fig. 1. Chemical structures of isolated compounds (**1**-**15**) from rhubarb

Desoxyrhapontigenin (6)
Brown powder; mp 175-177°C; UV λ_{max} (MeOH): 218, 302 and 322 nm; IR ν_{max} (KBr) cm^{-1}: 3430; 1580 and 1510; ^{1}H-NMR (400 MHz, CD_3OD): δ: 3.78 (3H, s, 4'-OCH_3), 6.18 (1H, t, *J* = 2.0 Hz, H-4), 6.46 (2H, d, *J* = 2.0 Hz, H-2, 6), 6.97 (1H, d, *J* = 16.4 Hz, H-α), 6.83 (1H, d, *J* = 16.4 Hz, H-β), 6.88 (2H, d, *J* = 8.8 Hz, H-3', 5'), 7.42 (1H, d, *J* = 8.8 Hz, H-2', 6'); ^{13}C-NMR (100 MHz, CD_3OD) δ: 160.9 (C-4'), 159.8 (C-3, 5), 141.3 (C-1), 131.6 (C-1'), 129.2 (C-α), 128.8 (C-2', 6'), 127.9 (C-β), 115.2 (C-3', 5'), 105.9 (C-2, 6) 102.9 (C-4), 55.8 (4'-OCH_3).

Emodin (7)
Yellow powder; mp: 267-268°C; UV λ_{max} (MeOH): 202, 208 and 218 nm; IR (KBr) ν_{max} cm^{-1}: 3430, 1640, 1560, 1480, 1340 and 1260; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.24 (1H, brs, H-4), 6.91 (1H, d, *J* = 2.0 Hz, H-5), 6.75 (1H, brs, H-2), 6.29 (1H, d, *J* = 2.0 Hz, H-7), 2.47 (3H, s, 3-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 190.9 (C-9), 181.5 (C-10), 166.0 (C-6), 165.4 (C-8), 162.4 (C-1), 148.8 (C-3), 135.9 (C-10a), 133.6 (C-4a), 124.2 (C-2), 120.8 (C-4), 113.8 (C-9a), 109.6 (8a), 109.1 (C-5), 108.1 (C-7), 21.3 (3-CH_3).

Rhapontigenin (8)
Amorphous powder; mp: 189-190°C; IR (KBr) λ_{max} cm^{-1}: 3300, 1620, 1580, 152, 1330, 1260, 1160; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.83 (3H, s, 4'-OCH_3), 6.18 (1H, t, *J* = 2.0 Hz, H-4), 6.45 (2H, d, *J* = 2.0 Hz, H-2, 6), 6.78 (1H, d, *J* = 16.4 Hz, H-α), 6.83 (1H, d, *J* = 16.4 Hz, H-β), 6.93 (1H, d, *J* = 7.5 Hz, H-5'), 6.88 (1H, dd, *J* = 2.1, 7.5 Hz, H-6'), 7.0 (1H, d, *J* = 2.1 Hz, H-2'); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 159.9 (C-3, 5), 149.7 (C-4'), 147.8 (C-3'), 141.2 (C-1), 132.3 (C-1'), 129.5 (C-α), 127.9 (C-β), 120.1 (C-2), 113.7 (C-3'), 112.8 (C-6'), 106.0 (C-2, 6), 102.9 (C-4), 56.5 (4'-OCH_3).

Chrysophanol-8-*O*-β-D-glucopyranoside (9)
Yellow powder; mp: 239-240°C; UV λ_{max} (MeOH): 202, 208 and 225 nm; IR (KBr) ν_{max} cm^{-1}: 3350, 2925, 1660, 1620, 1580, 1450, 1360, 1310, 1260, 1160; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.82 (1H, d, *J* = 7.8 Hz, H-7), 7.73 (1H, dd, 7.8, 8.4 Hz, H-6), 7.69 (1H, d, *J* = 8.4 Hz, H-5), 7.46 (1H, brs, H-2), 7.16 (1H, brs, H-4), Glu: 5.12 (1H, d, *J* = 7.4 Hz, H-1'), 3.93 (2H, dd, *J* = 2.4, 12.0 Hz, H-6"), 3.80 (1H, m, H-3"), 3.71 (1H, m, H-5"), 3.48 (1H, m, H-4"), 3.36 (1H, m, H-2"), 2.4 (3H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 187.4 (C-9), 181.6 (C-10), 161.5 (C-1), 157.8 (C-8), 147.2 (C-3), 135.5 (C-6), 134.3 (C-10a), 131.7 (C-4a), 123.6 (C-4), 122.1 (C-5), 118.9 (C-2), 120.1 (C-7), 116.3 (C-8a), 114.3 (C-9a), 21.1 (CH_3), Glu: 100.2 (C-1'), 76.1 (C-5'), 72.9 (C-2'), 76.9 (C-3'), 69.1 (C-4'), 60.2 (C-6').

Piracetannol-3'-O-β-D-glucopyranoside (10)
Amorphous powder; mp: 228-232°C, UV λ_{max} (MeOH): 221, 304 and 319 nm; IR (KBr) ν_{max} cm^{-1}: 3300 (OH), 1620, 1580, 1520 (aromatic C=C), 1330, 1260, 1160; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.45 (2H, d, *J* = 2.4 Hz, H-2'), 7.07 (1H, d d, *J* = 2.4, 8.4 Hz, H-6') 6.94 (1H, d, *J* = 16.4 Hz, H-α), 6.84 (1H, d, *J* = 16.4, H-β), 6.82 (1H, d, *J* = 8.4 Hz, H-5'), 6.45 (2H, d, *J* = 2.4 Hz, H-2, 6), 6.17 (1H, t, *J* = 2.0 Hz, H-4), 4.81 (1H, d, *J* = 7.2 Hz, H-1"), 3.96 (1H, dd, *J* = 2.4, 12.0 Hz, H-6"), 3.41-3.72 (4H, m, H-2" to H-5"); ^{13}C-NMR (100 MHz, CD_3OD) δ: 159.7 (C-3,5), 148.4 (C-3'), 147.2 (C-4'), 141.3 (C-1), 131.5 (C-1'), 129.3 (C-α), 128.0 (C-β), 123.7 (C-6'), 117.3 (C-5'), 116.8 (C-2'), 106.0 (C-2, 6), 104.7 (C-4), Glu: 102.9 (C-1"), 78.6 (C-5"), 77.8 (C-2"), 75.1 (C-3"), 71.6 (C-4"), 62.7 (C-6").

Piceid (11)
Amorphous powder; mp: 226-229°C; UV λ_{max} (MeOH): 221, 304 and 320 nm; IR (KBr) ν_{max} cm^{-1}: 3340, 1620, 1570, 1510, 1330, 1260, 1160; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.36 (2H, brd, *J* = 8.8 Hz, H-2', 6'), 7.01 (1H, d, *J* = 16.4 Hz, H-α), 6.84 (1H, d, *J* = 16.4, H-β), 6.78 (2H, dd, *J* = 1.6, 8.8 Hz, H-3', 5'), 6.76 (1H, brs, H-6), 6.62 (1H, brs, H-2), 6.45 (1H, t, *J* = 1.6 Hz, H-4); glu: 4.90 (1H, d, *J* = 7.6 Hz, H-1"), 3.93 (2H, dd, *J* = 2.0, 12.0 Hz, H-6"), 3.41-3.72 (4H, m, H-2" to H-5"); ^{13}C-NMR (100 MHz, CD_3OD) δ: 160.6 (C-3), 159.7 (C-5), 158.7 (C-4'), 141.6 (C-1), 130.5 (C-1'), 130.1 (C-α), 129.1 (C-2', 6'), 126.8 (C-$\hat{a}$), 116.6 (C-3', 5'), 108.5 (C-6), 107.2 (C-2), 104.2 (C-4), Glu:102.5 (C-1"), 78.3 (C-5"), 78.1 (C-2"), 75.1 (C-3"), 71.6 (C-4"), 62.7 (C-6").

ε-viniferin (12)
Pale brown powder; mp: 149-151°C; $[\alpha]_D^{25}$ -47.0° (*c*: 0.5, MeOH); UV λ_{max} (MeOH): 220, 304 and 319 nm; IR ν_{max} (KBr) cm^{-1}: 3340, 1610, 1570, 1510, 1330, 1260, 1160; ^{1}H-NMR (400 MHz, accetone-d_6) δ: 7.21 (2H, d, *J* = 8.4 Hz, H-2', 6'), 7,17 (2H, d, *J* = 8.4 Hz, H-2,6), 6.91 (1H, d, *J* = 16.2 Hz, H-7'), 6.83 (2H, d, *J* = 8.7 Hz, H-3', 5'), 6.74 (2H, d, *J* = 8.7 Hz, H-3, 5), 6.74 (1H, overlap, H-14'), 6.71 (1H, d, *J* = 16.2 Hz, H-8'), 6.33 (1H, d, *J* = 2.1 Hz, H-12'), 6.25 (3H, br s, H-10, 12, 14). ^{13}C-NMR (100 MHz, acetone-d_6) δ: 162.5 (C-4'), 159.9 (C-11, 13), 159.6 (C-11'), 157.7 (C-4, 13') 147.5 (C-9), 136.5 (C-9'), 133.9 (C-1), 130.2 (C-7'), 129.9 (C-1'), 128.7 (C-2', 6'), 128.0 (C-2, 6), 123.6 (C-8'), 119.9 (C-10'), 116.4 (C-3, 5), 116.2 (C-3', 5'), 107.1 (C-10, 14), 104.3 (C-14'), 102.2 (C-12), 96.9 (C-12'), 93.9 (C-7), 57.2 (C-8).

Ampelopsin B (13)
Pale brown powder; mp: 205-207°C; $[\alpha]_D^{25}$ +170.4° (*c* 0.10, MeOH); UV λ_{max} (MeOH): 224 and 281 nm; IR ν_{max} (KBr) cm^{-1}: 3340, 1590, 1510, 1150 and 970; ^{1}H-NMR (400 MHz, accetone-d_6) δ: 7.09 (2H, d, *J* = 8.7 Hz, H-1, 6), 6.93 (2H, d, *J* = 8.4 Hz, H-2', 6'), 6.76 (2H, d, *J* = 8.4 Hz, H-3', 5'),

6.63 (2H, d, *J* = 8.7 Hz, H-3,5), 6.42 (1H, d, *J* = 2.1 Hz, H-10), 6.05 (1H, d, *J* = 2.1 Hz, H-12), 5.72 (1H, d, *J*= 11.4 Hz, H-7), 5.21 (1H, t, *J* = 3.9 Hz, H-7'), 4.17 (1H, d, *J* = 11.4 Hz, H-8), 3.57 (1H, dd, *J* = 3.9, 17.5 Hz, H-8'), 3.20 (1H, brd, *J* = 17.4 Hz, H-8'). ^{13}C-NMR (100 MHz, acetone-d_6) δ: 160.5 (C-13'), 158.8 (C-4), 158.6 (C-13), 157.2 (C-11'), 156.7(C-13), 156.1 (C-11), 142.7 (C-9'), 138.2 (C-1'), 134.8 (C-9), 131.1 (C-1), 130.1 (C-2', 6'), 128.6 (C-2, 6), 122.9 (C-14), 119.1 (C-10'), 116.1 (C-3, 5), 115.6 (C-3', 5'), 109.1 (C-14'), 105.5 (C-10), 101.6 (C-12), 95.8 (C-12'), 88.4 (C-7), 49.4 (C-8), 35.9 (C-7´), 33.9 (C-8').

Isorhapontin (14)

Brown crystals; mp: 194-197°C; UV λ_{max} (MeOH): 221, 302 and 320 nm; IR ν_{max} (KBr) cm^{-1}: 3350, 1620, 1580, 1510, 1330, 1260, 1150; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.01 (1H, d, *J* = 1.6 Hz, H-2'), 6.95 (1H, brd, *J* = 8.4 Hz, H-6'), 6.92 (1H, d, *J* = 16.4 Hz, H-α), 6.89 (1H, d, *J* = 8.4 Hz, H-5´), 6.83 (1H, d, *J* = 16.4 Hz, H-β), 6.72 (1H, brs, H-2), 6.58 (1H, brs, H-6), 6.34 (1H, brs, H-4), 3.86 (OCH_3), Glu: 4.89 (1H, d, *J* = 7.2 Hz, H-1"), 3.93 (2H, dd, *J* = 2.4, 12.0 Hz, H-6"), 3.80 (1H, m, H-3"), 3.71 (1H, m, H-5"), 3.48 (1H, m, H-4"), 3.36 (1H, m, H-2"); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 160.6 (C-3), 159.8 (C-5), 149.2 (C-4'), 147.8 (C-3'), 141.3 (C-1), 132.2 (C-1'), 130.1 (C-α), 127.6 (C-β), 120.3 (C-6'), 113.8 (C-2'), 112.8 (C-5'), 108.5 (C-6), 107.2 (C-2), 104.3 (C-4), 102.5 (C-1"), 78.2 (C-5"), 75.1 (C-2"), 71.6 (C-4"), 62.7 (C-6"), 56.5 (OCH_3).

Emodin-8-β-D-glucopyranoside (15)

Yellow powder; mp: 220-221°C; UV λ_{max} (MeOH): 202, 208 and 218; IR (KBr) ν_{max} cm^{-1}: 3340, 1660, 1620, 1580, 1450. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.67 (1H, br s, H-4), 7.52 (1H, br s, H-2), 7.06 (1H, d, *J* = 2.8 Hz, H-5), 6.58 (1H, d, *J* = 2.4 Hz, H-7), Glu: 4.63 (1H, d, *J* = 7.2 Hz, H-1"), 3.72 (1H, dd, *J* = 4.0, 10.8 Hz, H-6"), 3.52 (1H, m, H-3"), 3.49 (1H, m, H-6"), 3.48 (1H, m, H-5"), 3.43 (1H, m, H-4"), 3.36 (1H, m, H-2"). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 186.2 (C-9), 182.1 (C-10), 164.5 (C-6), 164.2 (C-8), 158.3 (C-1), 134.3 (C-4a), 134.1 (C-10a), 121.5 (C-4), 118.4 (C-2), 108.2 (C-5), 107.1 (C-7), 100.7 (C-1'), 77.3 (C-5'), 76.5 (C-3'), 73.4 (C-2'), 69.6 (C-4'), 60.6 (C-6').

In Vitro lipoxygenase inhibition assay

Lipoxygenase inhibition activity was measured as previously reported with some modifications (Aziz *et al*, 2004). The reaction mixture contained 470 μL of 20 mM sodium phosphate buffer (pH 8.0), 10 μL of test compound, 10 μL linoleic acid solution (final concentration 40 μM), and 10 μL soybean lipoxygenase (100.000 U/mL) was added to initiate the reaction. The resulting solution was mixed well, and incubated at room temperature. After 5 min, the change of absorbance at 234 nm was read to measure conjugated diene produced. The inhibitory effect of compound on enzyme was calculated as:

$$\text{Inhibition (\%)} = [(Ac - As)/Ac] \times 100$$

Where Ac and As were absorbances of the control (without test sample) and the sample, respectively. Inhibitory activity was determined as a mean of triplicate measurements and expressed as the 50% inhibition concentration IC_{50} value from the control without inhibitor.

Lineweaver-Burk plots: The kinetic inhibition was carried out in the absence and presence of active compounds with various concentrations of linoleic acid as substrate. The initial rate was determined on the basis of the rate of increase in absorbance at 234 nm, which expressed the formation of conjugated diene hydroperoxide (13-HPOD, ε = 25 mM^{-1} cm^{-1}) (Tae *et al.,* 2004). The Michaelis constant (K_m) and maximal velocity (V_{max}) of the lipoxygenase were determined by Lineweaver-Burk plot analysis for competitive inhibition, and intercept on vertical axis for noncompetitive inhibition.

ABTS$^{\bullet+}$ radical scavenging assay

ABTS$^{\bullet+}$ radical scavenging assay was measured as previously reported (Re *et al.*, 1999). In briefly, ABTS$^{\bullet+}$ radical cation was produced by adding 400 μL of 60 mM $K_2S_4O_8$ (final concentration 2.45 mM) in 10 mL of 7mM ABTS solution. The mixture was kept in the dark at room temperature for 16 h for the examining of antioxidant compounds. The ABTS$^{\bullet+}$ solution was diluted with H_2O until absorbance at 734 nm (0.700 ± 0.020). For scavenging assay, 990 μL of diluted ABTS$^{\bullet+}$ solution was added to 10 μL of test compounds or Trolox standards (final concentration 0-20 μM) in MeOH, the absorbance was read in ambient temperature at exactly 6 min after the initial mixing. Stock solutions of the compounds were prepared so that they produced 10-80% inhibition of the blank absorbance. The percentage decrease of the absorbance at 734 nm was calculated and plotted as a function of the concentration of the antioxidants and of Trolox for the standard. To calculate the Trolox equivalent antioxidant coefficient (TEAC), the slope of the plot of the percentage inhibition of absorbance versus concentration for antioxidant was divided by the slope the plot of Trolox. All determinations were carried out in triplicate.

RESULTS AND DISCUSSION

The results presented in Table I showed the inhibitory activity of the EtOH extract of rhubarb, and its soluble fractions on lipoxygenase. The EtOAc- and BuOH-soluble fractions showed considerable inhibitory effects with IC_{50} values of 26.7 and 42.9 μg/mL, respectively. Meanwhile, hexane-soluble fraction had no inhibition. Repeated column

Table I. Inhibitory effects on lipoxygenase of the EtOH extract of rhubarb and its soluble-fractions

Extract/fractions	IC_{50} (μg/mL) [a]
EtOH extract	66.2 ± 3.2
Hexane fraction	> 100
EtOAc fraction	26.7 ± 2.8
BuOH fraction	42.9 ± 3.1
Baicalein[b]	3.4 ± 1.3
(+)-catechin [b]	19.7 ± 2.8

[a] Values are mean ± SD of three individual experiments.
[b] Positive control (μM).

chromatography of soluble fractions obtained fifteen compounds (**1**-**15**) including **1-2** from hexane-soluble fraction, **3**-**13** from EtOAc-soluble fraction and **14**-**15** from BuOH-soluble fraction. On the basis of their physicochemical data, and by comparing with those published in the literature, isolated compounds (**1**-**15**) were identified as chyrsophanol (**1**) (Sang *et al*., 2005), physcion (**2**) (Carmo *et al*., 1981), desoxyrhaponticin (**3**) (Kashiwada *et al*., 1984), rhapontincin (**4**) (Yoshiki *et al*., 1984), resveratrol (**5**) (Jin *et al*., 2002), desoxyrhapontigenin (**6**) (Kashiwada *et al*., 1984), emodin (**7**) (Choi *et al*., 2005), rhapontigenin (**8**) (Kashiwada *et al*., 1984), chrysophanol-8-*O*-*β*-D-glucopyranoside (**9**) (Kashiwada *et al*., 1984), piracetannol-3'-*β*-D-glucopyranoside (**10**) (Kashiwada *et al*., 1984), piceid (**11**) (Kashiwada *et al*., 1984), *ε*-viniferin (**12**) (Ito *et al.,* 1999; Kim *et al.,* 1999), isorhapontin (**14**) (Artemnika *et al*., 2004), and emodin-8-*O*-*β*-glucopyranoside (**15**) (Zhang *et al*., 2005).

Compound **13** was obtained as brown powder with mp 205-207°C and $[\alpha]_D^{25}$ +170.4°. The UV spectrum with maximal absorption at 224 and 281 nm, and the IR spectrum with bands at 3340 (OH), 1590, 1510, 1150 and 970 cm^{-1} (aromatic C=C) of **13** indicated the presence of stilbene moiety (Kashiwada *et al*., 1984). The ^{1}H-NMR spectrum exhibited twelve aromatic protons including four broad doublets signals at δ 7.09 (2H, brd, *J* = 8.7, H-2, 6), 6.93 (2H, brd, *J* = 8.4, H-2', 6'), and 6.67 (2H, brd, *J* = 8.4 Hz, H-3', 5'), and two pair doublet peaks at δ 6.42 and 6.05 (1H each, d, *J* = 2.1 Hz, H-10, 12), 6.32 and 6.22 (1H each, d, *J* = 2.1 Hx, H-12', 14'). One methylene protons signal was observed at δ 3.57 (1H, dd, *J* = 3.9, 17.4 Hz, H-8a') and 3.20 (1H, brd, *J* = 17.4 Hz, H-8b'). Two aliphatic methines δ 5.21 (1H, t, *J* = 3.9 Hz, H-7') and 4.17 (1H, d, *J* = 11.4 Hz, H-8), and an oxygenated methine at δ 5.72 (1H, d, *J* = 11.4 Hz, H-7) was also assigned. The ^{13}C-NMR spectra showed twenty eight carbon signals comprising one oxygenated methine carbon at δ 88.4 (C-7), one aliphatic methylene carbon at δ 33.9 (C-8'), two aliphatic methine carbons at δ 35.7 (C-7') and 49.4 (C-8), together with six quaternary carbons at δ 131.1 (C-1), 134.8 (C-9), 122.9 (C-14), 138.2 (C-1'), 142.7 (C-9'), and 119.1 (C-10'), six oxygenated aromatic carbons at δ 158.8 (C-4), 156.2 (C-11), 158.6 (C-13), 156.6 (C-4'), 157.2 (C-11') and 160.5 (C-13'), and twelve aromatic carbons. These data alluded that **13** was a dimer resveratrol derivative, and in comparison with those of published data and the structure of **13** was identified as ampelopsin B (Keckei *et al*., 2000; Takaya *et al.,* 2002). This is the first occurrence of ampelopsin B from rhubarb.

Table II. *In vitro* quantitative inhibition of lipoxygenase by isolated compounds (**1**–**15**)

Compounds	% Inhibitions[a] (100 μM)	IC_{50} (μM)[a]
Chrysophanol (**1**)	47.1 ± 2.1	ND
Physcion (**2***)*	17.7 ± 1.2	ND
Desoxyrhaponticin (**3**)	67.2 ± 2.1	74.1 ± 2.5
Rhaponticin (**4**)	87.5 ± 1.8	34.3 ± 1.5
Reveratrol (**5**)	92.4 ± 4.5	12.3 ± 2.5
Desoxyrhapotigenin (**6**)	80.3 ± 3.2	28.6 ± 3.7
Emodin (**7**)	31.4 ± 1.6	ND
Rhapontigenin (**8**)	98.2 ± 2.4	10.7 ± 1.2
Chrysophanol-8-*O*-*β*-D-Glc (**9**)	46.2 ± 2.3	ND
Piceatannol-3'-*O*- *β*-D-Glc (**10**)	65.9 ± 1.5	69.5 ± 2.4
Piceid (**11**)	74.8 ± 4.1	55.4 ± 4.5
ε-viniferin (**12**)	98.2 ± 3.2	6.7 ± 1.9
Ampelopsin B (**13**)	95.4 ± 2.9	8.9 ± 2.1
Isorhaponticin (**14**)	76.2 ± 2.5	52.8 ± 2.3
Emodin-1-*O*-Glc (**15**)	25.7 ± 2.0	ND
Baicalein[b]	98.2 ± 2.1	3.4 ± 1.3
(+)-catechin[b]	92.8 ± 3.1	19.7 ± 2.8

[a] Values are the mean ± SD of triplicate experiments.
[b] Positive control.
ND: Not determined.

The inhibitory effects of the isolated compounds on the LOX are showed in Table II. Compounds **1**-**2**, **7**, **9** and **15** which possessed anthraquinone skeletons, exhibited weak or no inhibitory activity. Whereas, compounds (**3**-**6**, **8**, and **10**-**14**) which possessed stilbene skeletons displayed significant inhibition of the LOX activity with the IC_{50} values ranging from 6.7 to 74.1 μM compared with baicalein (IC_{50} = 3.4 μM) and (+)-catechine (IC_{50} = 19.7 μM), which used as positive controls (Aziz *et al*., 2004; Sadix *et al*., 2003; Banerjee, 2006; Yamamoto *et al*., 2005). Subsequently, the inhibition kinetics of soybean lipoxygenase-1 by resveratrol (**5**), *ε*-viniferine (**12**) and ampelopsin B (**13**) was performed. Under the conditions employed in the present investigation, the oxidation of linoleic acid catalyzed by LOX follows Michaelis-Menten kinetics. The kinetic parameters for this oxidase obtained from a Lineweaver-Burk plots show that *K*m values are equal to 54.44, 124.51 and

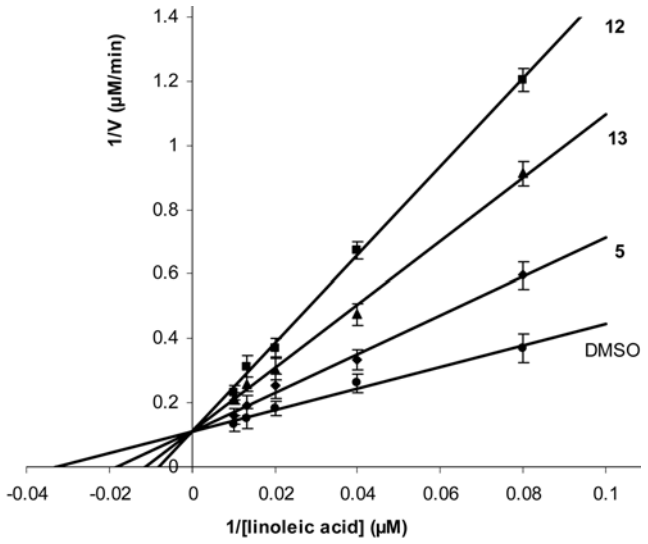

Fig. 2. Lineweaver-Burk plots of xanthine oxidase inhibitory activity. With linoleic acid as a substrate, in the presence of **5**, 10 μM (◆); **12**, 10 μM (■); **13**, 10 μM (▲); and DMSO (●) as a control. The data represent the mean ± S.D. of triplicate different experiments.

Table III. Scavenging activities against $ABTS^{\bullet+}$ of stilbene constituents

Compounds	TEAC [a, b]
3	1.16 ± 0.02
4	1.62 ± 0.05
5	3.52 ± 0.03
6	2.13 ± 0.04
8	3.28 ± 0.01
10	2.17 ± 0.03
11	1.78 ± 0.04
12	4.64 ± 0.02
13	4.21 ± 0.03
14	1.67 ± 0.05
Cafeic acid [c]	1.92 ± 0.08
BHT [c]	0.84 ± 0.02

[a] The values of scavenging activity against $ABTS^{\bullet+}$ were expressed as Trolox equivalent antioxidant capacity (TEAC), which is the concentration (mM) of Trolox having the same activity as 1 mM of sample.
[b] Values are mean ± SD of three experiments.
[c] Positive control.

89.55 μM of compounds **5**, **12** and **13**, respectively, and *V*max value is equal to 9.058 μM/min. As illustrated in Fig. 2, the inhibition kinetics analyzed by Linewearver-Burk plots showed that investigated compounds **5** (Tea *et al*., 2004) and **12**-**13** are competitive inhibitors because the presences of tested compounds resulted in a family of lines with a common intercept in the 1/V axis but with different slopes. This may suggest that stilbenes displace linoleic acid from the enzymatic site of oxidation.

Based on our results and the chemical structures, the isolated stilbenes showed significantly differences in the relationships with respect to their activities and chemical structures. Compounds **12** and **13** are dimer-stilbenes which possessed five hydroxy groups at C-4, C-11, C-13, C-4' and C-13', inhibited the lipoxygenase with IC_{50} values as 6.7 and 8.9 μM, respectively. The mono-stilbenes (**3**-**6**, **8**, **10**-**11** and **14**) in which were less active than dimer-stilbenes, because of not only less hydroxyl groups but also affected methoxy and glucose groups. The stilbene glucosides (**3**-**4**, **10**-**11** and **14**) exhibited lower inhibitory activity than the stilbenes (**5**-**6**, and **8**) with out glucose in the structures. Compound **5** (IC_{50} = 12.3 μM) which possessed a hydroxy group at C-4' were 2.3 fold inhibition activity than compound **6** (IC_{50} = 28.6 μM) with a methoxy group at the same position, when one more hydroxy group added in B-ring at C-3' of compound **8** (IC_{50} = 10.7 μM), its inhibitory effects increased. Interestingly, compound **4** with a hydroxy group at C-3' and a methoxy at C-4' exhibited stronger activity than compound **14** with a hydroxy group at C-4' and a methoxy group at C-3'. In addition, glucose attached to B-ring at C-3' of compound **10** (IC_{50} = 69.5 μM) exhibited significantly less inhibitory activities than compound **11** (IC_{50} = 55.4 μM) in which a glucose group attached to A-ring at C-5. These indicated that, the numbers and position of hydroxy and methoxy groups and glucose moieties influenced to their potent inhibitory effects on lipoxygenase.

Oxidative degradation of polyunsaturated fatty acids occurs in two sequential steps of initiation and propagation (Kubo *et al*., 2002). Lipoxygenase as a initiator involved in the first step of lipid peroxidation leading to hydroxyl radical ($OH^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), alkoxyl radical ($RO^{\bullet}$) or alkyl radical ($R^{\bullet}$) in the biological system. The propagation steps in lipid peroxidation have been known as the rapid reactions between the fatty acid alkyl radicals and the molecular oxygen (Jacob *et al*., 2001). We found that stilbene derivatives were significantly stable free radical scavenging activities against $ABTS^{\bullet+}$ with TEAC values ranging from 1.1 to 4.5 (Table III). Dimer-stilbenes (**12** and **13**) showed stronger scavenging activities than mono-stilbene compounds in which stilbene glycosides (**3**, **4**, **10**, **11** and **14**) exhibited less active than stilbenes (**5** and **8**). In this study, anthraquinone derivatives had no scavenging activity against $ABTS^{\bullet+}$ (data not shown). Thus, stilbene derivatives may be inhibited both the initiation and propagation steps in lipid peroxidation by inhibiting enzymes and scavenging free radicals.

In conclusion, the stilbenes and anthraquinones are the major constituent of rhubarb. Anthraquinone derivatives were neither inhibitory effect on lipoxygenase nor scavenging free radical against $ABTS^{\bullet+}$. All most stilbene

derivatives were considered to be main lipoxygenase inhibitors and free radical scavenger. From above results, we have found that dimer-stilbenes are good activity than mono-stilbenes, phenolic hydroxyl group at B-ring enhanced the activity and glucoside moiety reduces the activity. These results suggested that rhubarb may be beneficial in preventing related diseases in the ethno-medicine.

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REFERENCES

Artemkina, N. A. and Rhoschin, V. I., Extractive substances from needles and shoots of *Picea abies* (L.) Karst. 1. Phenolic compounds, their isolation and identification. *Rastitel'nye Resursy.,* 40, 77-87 (2004).

Aziz, R., Malik, A, Riaz, N., Nawaz, H. R., Ahmad, H, Nawaz, S. A., and Choudhary, M. I., Lipoxygenase inhibitory consitituents from *Periploca aphylla*. *J. Nat. Prod*., 67, 1450-1454 (2004).

Banerjee, S., Inhibition of mackerel (Scomber scombrus) muscle lipoxygenase by green tea polyphenols. *Food. Res. Int.*, 39, 486-491 (2006).

Bae, K., The medicinal plants of Korea. Kyo-Hak Publishing Co., Seoul, p. 90, (2001).

Carmo, M. D., Miraglia, M., Antonio, A. L., Mesquita, M., De Jesus, C., Varijao, Gottlieb, O. R., and Gottlieb, H. E., Anthraquinones from *Vismia* species. *Phytochemistry.*, 20, 2041-2042 (1981).

Gardner, H. W., Recent investigations into the lipoxygenase pathway of plants., *Biochim. Biophys. Acta*., 1084, 221-239 (1991).

Hisashi, M., Toshio, M., Iwao, T., Ji, Y. P., Shoichi, H., and Masayuki. Y., Antioxidant constituents from rhubarb: Structural requirements of stilbenes for the activity and structures of two new anthraquinone glucosisdes. *Bioorg. Med. Chem*., 9, 41-50 (2001).

Ito, J., Takaya, Y., Oshima, Y., and Niwa, M., New oligostilbenes having a benzofuran form Vitis vinifera Kyohou. *Tetrahedron*., 55, 2529-2544 (1999).

Jin, W. Y., Na, M. K., An, H. Y., Bae, K., and Kang, S. S., Antioxidant compounds from twig of *Morus alba*. *Nat. Pro. Sci*., 8, 129-132 (2002).

Kashiwada, Y., Nonaka, G. I., and Nishioka, I., Studies on rhubarb (Rhei rhizoma). Isolation and characterization of stilbenes. *Chem. Pharm. Bull*., 32, 3501-3517(1984).

Keckei, K., Sarker, S. D., and Dinan, L., Resveratrol-type oligostilbene from Iris clarkei antagonize 20-hydroxyecdysone action in the Drosophila melanogaster BII cell line. *Cell. Mol. Life Sci*., 57, 333-336 (2000).

Kim, H. J., Chung, S. K., and Choi, S. W., Lipoxygenase inhibitors from *Paeonia lactiflora* seeds. *J. Food Sci. Nutr.*, 4, 163-166 (1999).

Kubo, I., Masuoka, N., Xiao, P., and Haraguchi, H., Antioxidant activity of docecyl gallate. *J. Agric. Food Chem.,* 50, 3533-3539 (2002).

Nie, D. and Honn, K. V., Cyclooxygenase, lipoxygenase and tumor angionesis. *Cell. Mol. Life Sci*., 59, 799-807 (2002).

Re, R., Pellegrini, n., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C., Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med*., 26, 1231-1237 (1999).

Sadik, C. D., Sies. H., and Schewe, T., Inhibition of 15-lipoxygenase by flavonoids: structure activity relations and mode of action. *Biochem. Pharm*., 65, 773-781 (2003).

Sang, Z. C., Sung. O. L., Ki. U. C., Sang, H. P., Hee. C. K., Eun. Y. Y., Hi. J. C., and Kang. R. L., Antidiabetic stilbene and anthraquinone derivatives from *Rheum undulatum*. *Arch. Pharm. Res*., 28, 1027-1030 (2005).

Song, J, H., Yang, T. C., Chang, K. W., Han, S. K., Yi, H. K., and Jeon, J. G., In vitro anti-carcinogenic activity of dichloromethane fraction from *Rheum undulatum* L. root. *Arch. Pharm. Res*., 29, 490-496 (2006).

Tae J. H. and Isao. K., Lipoxygenase Inhibitory Activity of Anacardic Acids. *J. Agric. Food Chem*., 2005, 53, 4350-4354.

Takaya, Y., Yan, K. X., Terashima, K., Ito, J., and Niwa. M., Chemical determination of the absolute structures of resveratrol dimmers, amplelopsin A, B, D and F. *Tetrahedron*, 58, 7259-7265 (2002).

Yamamoto, S., Katsukawa, M., Nakano, A., Hiraki, E., Nishimura, K., Jisaka, M., Yokota, K., and Ueda, N., Arachidonate 12-lipoxygenases with reference to their selective inhibitors. *Biochem. Biophys. Res. Comm.,* 338, 122-127 (2005).

Zhang, X., Thuong, P. T., Jin, W. Y., Su, N. D., Sok, D. E., Bae, K., and Kang, S. S., Antioxidant activity of anthraquinones and flavonoids from flower of *Reynoutria sachalinensis*. *Arch. Pharm. Res*., 28, 22-27 (2005).