Low Density Lipoprotein-oxidation Inhibitory Phytochemicals from the Fruits of *Rhus parviflora*

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Abstract Fruits of *Rhus parviflora* were extracted with 80% aqueous methanol (MeOH), and the concentrated extract was partitioned using ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH), and H_2O, successively. Purification of EtOAc fraction led to isolation of fifteen polyphenols of which structures were identified by spectroscopic methods including 2D-NMR. Most compounds apart from compound **10** inhibited low density lipoprotein-oxidation within IC_{50} value of 10 μM. Among compounds, taxifolin (**2**), quercetin 3-*O*-α-L-rhamnopyranoside (**13**), agathisflavone (**5**) sulfuretin (**4**), and aureusidin (**3**) showed IC_{50} values 0.9, 0.8, 5.8, 2.9, and 2.4 μM which were of highly significant in comparison positive control butylated hydroxytoluene with IC_{50} value of 2.1 μM. The results indicate fruits of *R. parviflora* as a source of anti-hypercholesterolemic compounds.

Keywords anacardiaceae · anti-hypercholesterolemic · aurone · biflavonoid · low density lipoprotein-oxidation · *Rhus parviflora*

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R. parviflora Roxb. (Aanacardiaceae) is a sub-deciduous shrub of south Asia with distribution in Nepal, northern India, Bhutan and Sri Lanka (Press et al., 2000). Its edible berries which get ripe in December-February are used in Ayurvedic medicine for curing neurological and stomach disorders (Anonymous, 2006). We reported previously cytotoxic (Shrestha et al., 2012c), anti-insomnia (Shrestha et al., 2012b), neuroprotective (Shrestha et al. 2012a; 2013a; 2013c), anti-inflammatory (Shrestha et al. 2013b) properties of compounds from *R. parviflora* fruit. In this study we further evaluated the compounds for their low-density lipoprotein (LDL)-oxidation inhibition capacity.

Cardiovarsular disease is one of the leading causes of mortality with toll of 16.7 million deaths per year (Dahlöf, 2010). The oxidation of LDL is an early pathological process of atherosclerosis (Glass and Witztum, 2001). LDL particles are trapped in the intima, undergoes oxidative modifications caused by enzymatic attack of myeloperoxidase and lipoxygenases, or by reactive oxygen species during inflammation and atherosclerosis (Hansson and Hermansson, 2011).

The air-dried fruits (6 kg) of *R. parviflora* were extracted at room temperature in 80% aqueous MeOH (25 L ×3) for 24 h gave concentrated extract (1440 g). The MeOH extract was suspended in distilled H_2O (6 L) and successively partitioned with EtOAc (6 L ×3) and *n*-BuOH (6 L ×3), to obtain concentrated extracts in the EtOAc (RPE, 48 g), *n*-BuOH (RPB, 173 g), and H_2O (RPW, 1219 g) fractions.

The concentrated EtOAc fraction (RPE, 48 g) was applied to silica gel column chromatography (c.c.) (ϕ 14×12 cm) and eluted with *n*-hexane-EtOAc [(10:1→3:1→1:1, 20 L of each) and $CHCl_3$-MeOH (6:1→1:1, 10 L each)], gave 22 fractions. Fraction RPE-13 [2.41 g, elution volume/total volume (V_e/V_t) 0.59-0.80] was subjected to a Sephadex LH-20 c.c. (ϕ 2.5×45 cm), and eluted with MeOH-H_2O (2:1, 500 mL→2:1, 1.5 L) isolated fustin, (**1**) at RPE-13-6 [15.5 mg, V_e/V_t 0.02-0.04, TLC (RP-18 F_{254}s) R_f 0.87, in MeOH-H_2O (3:1)] and taxifolin (**2**) at RPE-13-7 [30.0 mg, V_e/V_t 0.04-0.07, TLC (RP-18 F_{254}s) R_f 0.85, in MeOH-H_2O (3:1)],
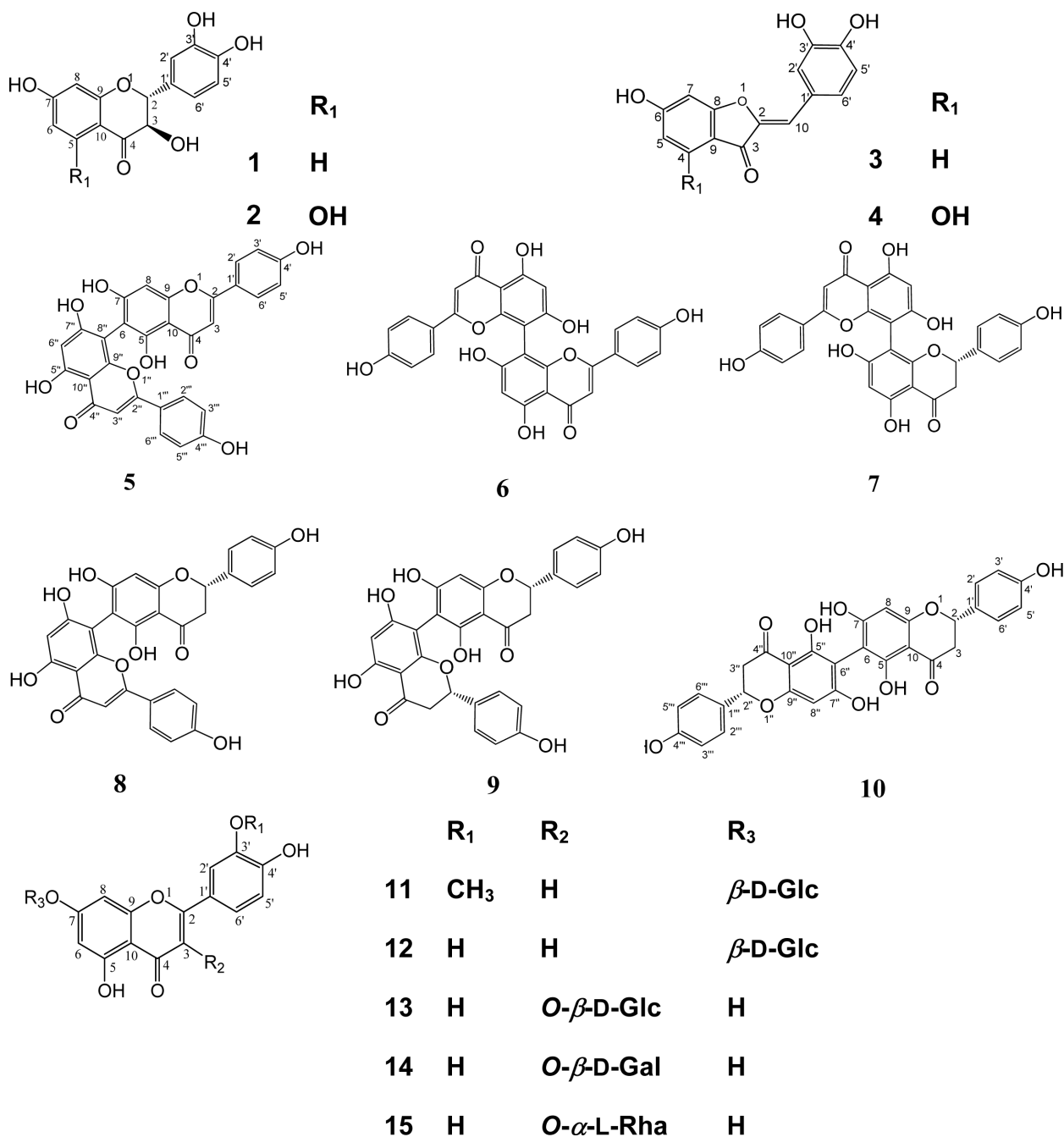
Fig. 1 Chemical structures of the compounds **1-15** from the fruits of *R. parviflora*.

succedaneaflavanone (**10**) at RPE-13-10 [11.7 mg, V_e/V_t 0.10–0.11, TLC (RP-18 $F_{254}S$) R_f 0.68 in MeOH-H_2O (3:1)] and mesuaferrone B (**7**) at RPE-13-17 [30 mg, V_e/V_t 0.59–0.80, TLC (RP-18 $F_{254}S$) R_f 0.55 in MeOH-H_2O (3:1)]. Fraction RPE-13-13 (475 mg, V_e/V_t 0.16–0.34) was applied to ODS c.c. (φ 3×4 cm), and eluted with MeOH-H_2O (2:3, 500 mL→2:1, 1.5 L) gave rhusflavanone (**9**) at RPE-13-13-8 [144 mg, V_e/V_t 0.17-0.22, TLC (RP-18 $F_{254}S$) R_f 0.45 in MeOH-H_2O (2:1)]. The RPE-15 fraction (2.33 g, V_e/V_t 0.52–0.54) was subjected to an ODS c.c. (φ 4×5 cm), and was

eluted with MeOH-H_2O (2:1, 4 L→1:1, 1 L) gave 13 fractions. The RPE-15-3 fraction (326.0 mg, V_e/V_t 0.01-0.09) underwent Sephadex LH-20 c.c. (φ 2×45 cm), and was eluted with MeOH-H_2O (1:1, 4.5 L), yielded aureusidin (**4**) at RPE-15-3-11 [18.4 mg, V_e/V_t 0.65–0.83, TLC (RP-18 $F_{254}S$) R_f 0.50, in MeOH-H_2O (2:1)]. The RPE15-4-3 fraction (88.7 mg, V_e/V_t 0.02–0.04) was subjected to the Sephadex LH-20 c.c. (φ 2.5×35 cm) and eluted with MeOH-H_2O (2:1, 1,200 mL, yielded sulfuretin (**3**) at RPE-15-4-13-3 [5.6 mg, V_e/V_t 0.25–0.41, TLC (RP-18 $F_{254}S$) R_f 0.45 MeOH-H_2O

Table 1 Inhibition activity of compounds (1-15) from the fruits of *R. parviflora* on LDL-oxidation[1]

Compounds	LDL-oxidation Inhibition (%)		IC$_{50}$ (μM)
	100 μM/ml	10 μM/ml	
fustin (**1**)	92.6±0.6	98.5±1.4	2.4
taxifolin (**2**)	99.7±0.2	91.3±2.2	0.9
sulfuretin (**3**)	97.0±0.2	101.8±0.9	2.9
aureusidin (**4**)	97.5±0.1	99.8±0.2	2.4
agathisflavone (**5**)	99.7±0.2	81.5±3.1	5.8
cupressuflavone (**6**)	99.3±0.7	68.5±1.6	7.3
mesuaferrone B (**7**)	93.5±0.2	75.8±1.2	8.4
rhusflavone (**8**)	97.0±0.0	51.6±4.7	9.0
rhusflavanone (**9**)	99.2±0.3	57.4±3.0	10.5
succedaneaflavanone (**10**)	87.6±0.6	14.5±0.3	58.2
chrysoeriol-7-*O*-β-D-glucopyranoside (**11**)	96.3±0.1	72.9±1.0	7.8
luteolin-7-*O*-β-D-glucopyranoside (**12**)	97.4±0.0	94.7±1.4	1.6
quercetin 3-*O*-β-D-glucopyranoside (**13**)	98.4±0.5	94.7±0.2	1.5
quercetin 3-*O*-β-D-glactopyranoside (**14**)	97.8±0.9	85.9±0.6	1.6
quercetin 3-*O*-α-L-rhamnopyranoside (**15**)	98.3±0.3	101.8±0.2	0.8

Data are means ± SD (n=3).
[1]Positive control of LDL-Oxidation, BHT, showed IC$_{50}$ value of 2.1 μM.

(2:1)]. The RPE-15-5 fraction (461.6 mg, V$_e$/V$_t$ 0.32–0.45) underwent the Sephadex LH-20 c.c. (φ 2.5×45 cm), and was eluted with MeOH-H$_2$O (2:1, 6 L), gave 13 fractions. Fraction RPE-15-5-10 (81 mg, V$_e$/V$_t$ 0.53–0.67) was subjected to ODS c.c. (φ 2.5×3 cm) and eluted with MeOH-H$_2$O (2:3, 250 mL→2:1, 300 mL) gave rhusflavone (**8**) at RPE-15-5-10-6 [66 mg, V$_e$/V$_t$ 0.35–0.46, TLC (RP-18 F$_{254}$S) R$_f$ 0.55 in MeOH-H$_2$O (3:1)]. Fraction RPE-15-5-12 (71.4 mg, V$_e$/V$_t$ 0.83–0.96) was subjected to the ODS c.c. (φ 1.2×4 cm) and eluted with MeOH-H$_2$O (1:2, 200 mL), isolated agathisflavone (**5**) at RPE-15-5-12-3 [60 mg, V$_e$/V$_t$ 0.24-1.0, TLC (RP-18 F$_{254}$S) R$_f$ 0.30 MeOH-H$_2$O (2:1)]. Fraction RPE-15-6 (708 mg, V$_e$/V$_t$ 0.45–0.87) subjected to Sephadex LH-20 column (φ 2.5×45 cm) and eluted with MeOH-H$_2$O (2:1, 5 L), led to isolation of cupressuflavone (**6**) at RPE-15-6-6 [19 mg, V$_e$/V$_t$ 0.30–0.36, TLC (RP-18 F$_{254}$S) R$_f$ 0.30 in MeOH-H$_2$O (3:1)].

Further, fraction RPE-17 [2 g, V$_e$/V$_t$ 0.56–0.60] was subjected to a Sephadex LH-20 c.c. (φ 2.5×45 cm), and eluted with MeOH-H$_2$O (1:1, 4 L), isolated chrysoeriol-7-*O*-β-D-glucopyranoside (**11**) at RPE-17-21 [44.0 mg, V$_e$/V$_t$ 0.40–0.42, TLC (RP-18 F$_{254}$S) R$_f$ 0.62 in MeOH-H$_2$O (2:1)]. Fraction RPE-18 (2 g, V$_e$/V$_t$ 0.60–0.61) purified by Sephadex LH-20 c.c. (φ 2.5×45 cm), and eluted with MeOH-H$_2$O (1:1, 2 L), resulted in 16 fractions. Fraction RPE-18-8 (90 mg, V$_e$/V$_t$ 0.09–0.16) was subjected to the SiO$_2$ c.c. (φ 2×10 cm), and eluted with CHCl$_3$-MeOH (6:1, 300 mL→3:1, 600 mL), isolated luteolin-7-*O*-β-D-glucopyranoside (**12**) at RPE-18-8-6 [7.5 mg, V$_e$/V$_t$ 0.13-0.21, TLC (SiO$_2$ F$_{254}$) R$_f$ 0.55 in CHCl$_3$-MeOH (5:1)]. The fraction RPE-18-9 (239 mg, V$_e$/V$_t$ 0.16–0.24) subjected to the SiO$_2$ c.c. (φ 3×10 cm), and eluted with CHCl$_3$-MeOH (7:1, 200 mL→6:1, 700 mL→5:1, 700 mL→4:1, 500 mL→1:1, 500 mL), isolated quercetin-3-*O*-β-D-glucopyranoside (**13**) at RPE-18-9-4 [21.9 mg, V$_e$/V$_t$ 0.04–0.06, TLC (SiO$_2$ F$_{254}$) R$_f$ 0.57 in CHCl$_3$-MeOH-H$_2$O (6:4:1)]. The fraction RPE-18-10 (510 mg, V$_e$/V$_t$ 0.24–0.39) subjected to the Sephadex LH-20 c.c. (φ

2.5×40 cm), and eluted with MeOH-H$_2$O (1:3, 15 L) to provided 18 fractions, and isolated quercetin-3-*O*-β-D-galactopyranoside (**14**) at RPE-18-10-4 [45.7 mg, V$_e$/V$_t$ 0.17–0.19, TLC (RP-18 F$_{254}$S) R$_f$ 0.55 in MeOH-H$_2$O (2:1)]. Fraction RPE-18-10-11–13 [81 mg, V$_e$/V$_t$ 0.36–0.77] was subjected to the SiO$_2$ c.c. (φ 2.2×10 cm), and eluted with CHCl$_3$-MeOH-EtOH-H$_2$O (34:3:3:2, 3 L) gave quercetin-3-*O*-α-L-rhamnopyranoside (**15**) at RPE-18-10-11–13-6 [34 mg, V$_e$/V$_t$ 014–0.29, TLC (RP-18 F$_{254}$) R$_f$ 0.62 in MeOH-EtOH-H$_2$O (9:1:1)].

The structures of isolated compounds **1-15** (Fig. 1) were determined by spectroscopic techniques and by comparison of their spectral data with literature values, and were reported previously by us (Shrestha et al., 2012a; 2012b; 2012c; Shrestha et al., 2013a; 2013b; 2013c). LDL-oxidation assay was carried out using the method described previously (Lee et al., 2009), with butylated hydroxytoluene (BHT) as a positive control. The data of assay are expressed as mean ± SD of three replicated experiments (Table 1).

Among the compounds evaluated, several compounds showed competent potency of inhibitory activity on LDL oxidation in comparison to positive control BHT (IC$_{50}$ value 2.1 μM). Taxifolin (**2**) showed significant inhibition of LDL-oxidation (IC$_{50}$ value 0.9 μM), the compound was reported previously for its inhibitory effect on hepato-cellular cholesterol biosynthesis in HepG2 cells (Gebhardt, 2003) and inhibition of LDL cholesterol via scavenging of MPO-derived NO$_2$ radicals (Kostyuk et al., 2003). Further, the LDL inhibitory potential of compounds fustin (**1**), aureusidin (**4**) and sulfuretin (**3**) with IC$_{50}$ values of 2.0, 2.9 and 2.4 μM are noteworthy. The extract of *Rhus verniciflua* with compounds fustin and sulfuretin had been reported for preventing and treating cholesterolemia, hyperlipidemia and fatty liver (Na et al., 2013). The biflavonoids, agathisflavone (**5**), rhusflavone (**8**), cupressuflavone (**6**), rhusflavanone (**9**), mesuaferrone B (**7**) and succedanea-

flavanone (**10**) had IC$_{50}$ values of 5.8, 7.3, 8.4, 9.0, 10.5 and 58.2 μM respectively. Biflavonoids with both units flavanone had comparatively low inhibitory capacity than those with both units flavone or combination of flavone and flavanone. A bioflavonoid, morelloflavone had been reported for inhibition HMG-CoA reductase (the rate-limiting enzyme) of the cholesterol biosynthetic pathway (Tuansulong et al., 2011). The flavonoid glycosides with the potent inhibition capacity of LDL-oxidation were quercetin 3-*O*-α-L-rhamnopyranoside (**15**), quercetin 3-*O*-β-glucopyranoside (**13**), quercetin 3-*O*-β-glactopyranoside (**14**), luteolin-7-*O*-β-D-glucopyranoside (**12**), and chrysoeriol-7-*O*-β-D-glucopyranoside (**11**) with IC$_{50}$ value of 0.8, 1.5, 1.6, 1.6 and 7.7 μM, respectively. Hou et al. (2004) and Shabana et al. (2007) had reported LDL-oxidation inhibitory potential of flavonoid glycosides (**11-15**) and orthodihydroxyl group is regarded as responsible for inhibitory effect (Hou et al., 2004). The inhibitory capacity of bioflavonoids (**5-10**) and an aurone, aureusidin (**4**) are reported here for the first time. The anti-hypercholesterolemic effect of biflavonoids by inhibition of LDL oxidation indicates cardiovascular protective anti-hypercholesterolemic potential of *R. parviflora* fruit.

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