# Antioxidative Phenolics from the Petals of *Carthamus tinctorius*

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The petals of safflower (*Carthamus tinctorius* L., Compositae) have been used as a source of folk medicine to promote blood circulation by removing the blood stasis [Yin and He, 2000] and as natural red and yellow colorants for dyeing fabrics, food colorings, and cosmetics [Obara and Onodera, 1979; Kim and Paik, 1997; Cho *et al.*, 2000; Yoon *et al.*, 2003]. The structures of these red and yellow colorants from safflower are reported to contain *C*-glucosyquinochalcone moieties [Kazuma *et al.*, 2000]. Besides *C*-glucosylquinochalcones, safflower petals also have flavonoid glycosides [Kazuma *et al.*, 2000; Hattori *et al.*, 1992]. Flavonoids are naturally occurring polyphenolics with antioxidant activities proposed to contribute to the human health.

From the EtOAc–soluble fraction of the MeOH extract of dried safflower petals, we isolated five flavonol glycosides and caffeic acid. The radical-scavenging activities of the compounds against ABTS radical are described in this paper.

The petals of *C. tinctorius* (450 g) were extracted at room temperature with MeOH (2 L × 2). After filtration and evaporation of the solvent under reduced pressure, the combined crude methanolic extract was suspended in water and then successively partitioned with EtOAc and BuOH to afford EtOAc-soluble (13.5 g) and BuOH-soluble (12.7 g) fractions. The EtOAc-soluble fraction was subjected to Sephadex LH-20 (6 × 30 cm) using MeOH/$H_2O$ gradient to yield four fractions. Further purification of these fractions using the reversed phase HPLC (20~40% $CH_3CN$/0.1% TFA-$H_2O$, ODS column; 250 × 10 mm, flow rate; 1 mL/min) yielded five flavonol glycosides (6-hydroxykaempferol 3-*O*-glucoside **1**, quercetin 3-*O*-rutinoside, quercetin 3-*O*-glucoside **2**, kaempferol 3-*O*-rutinoside, kaempferol 3-*O*-glucoside **3**) with the retention times of 24.3, 27.8, 31.3, 31.7, and 35.3 min, respectively, and caffeic acid **4** with retention time of 26.5 min (Fig. 1). Identification of these phenolics from the EtOAc-soluble fraction of the safflower was determined using the spectroscopic methods as described below. Among the identified phenolics, quercetin 3-*O*-rutinoside and kaempferol 3-*O*-rutinoside have also been isolated from the BuOH-soluble fraction of same plant and their spectroscopic data and antioxidative activities reported [Yoon *et al.*, 2007].

Compound **1** (13.1 mg): $R_t$ 24.3 min; UV (MeOH) 339, 279 nm; IR (KBr) 3301, 1659, 1609 $cm^{-1}$; LCMS *m/z* 487.4 $[M+Na]^+$, 465.4 $[M+H]^+$, 303.3 $[M-162+H]^+$; $^1H$ NMR data, see Table 1; $^{13}C$ NMR data, see Table 2. On the basis of $^1H$-, $^{13}C$-, and 2D-NMR data, compound **1** was determined to be 6-hydroxykaempferol 3-*O*-glucoside [Hattori *et al.*, 1992; Kazuma *et al.*, 2000].

Compound **2** (11.3 mg): $R_t$ 31.3 min; UV (MeOH) 366, 259 nm; IR (KBr) 3315, 1655, 1603 $cm^{-1}$; LCMS *m/z* 487.2 $[M+Na]^+$, 465.2 $[M+H]^+$, 303.2 $[M-162+H]^+$; $^1H$ NMR data, see Table 1; $^{13}C$ NMR data, see Table 2. The chemical structure of **2** was determined to be quercetin 3-*O*-glucoside on the basis of $^1H$-, $^{13}C$-, and 2D-NMR data.

Compound **3** (12.4 mg): $R_t$ 35.3 min; UV (MeOH) 348, 266 nm; IR (KBr) 3315, 1656, 1607 $cm^{-1}$; LCMS *m/z* 471.2 $[M+Na]^+$, 449.3 $[M+H]^+$, 287.3 $[M-162+H]^+$; $^1H$ NMR data, see Table 1; $^{13}C$ NMR data, see Table 2. On the basis of NMR data, compound **3** was established as kaempferol 3-*O*-glucoside [Kazuma *et al.*, 2000].

Compound **4** (8.7 mg): $R_t$ 26.5 min; UV (MeOH) 328, 303 (sh), 243, 220 nm; IR (KBr) 3030, 1660, 1594 $cm^{-1}$; LCMS *m/z* 181.1 $[M+H]^+$; $^1H$ NMR ($CD_3OD$) δ 7.52 (1H, *d*, *J* = 16.0 Hz, H-7), 7.02 (1H, *d*, *J* = 2.0 Hz, H-2), 6.92 (1H, *dd*, *J* = 8.4, 2.0 Hz, H-6), 6.77 (1H, *d*, *J* = 8.4 Hz, H-5), 6.21 (1H, *d*, *J* = 16.0 Hz, H-8); $^{13}C$ NMR ($CD_3OD$) δ 170.84 (C-9), 149.28 (C-4), 146.89 (C-7), 146.62 (C-3), 127.67 (C-1), 122.73 (C-6), 116.38 (C-5), 115.40 (C-8), 114.99 (C-2). The chemical structure of compound **4** was determined to be caffeic acid on the basis of the authentic compound obtained commercially and the NMR data [Flamini *et al.*, 2001].

Measurement of the radical-scavenging activity of compounds **1-4** was carried out using the decolorization

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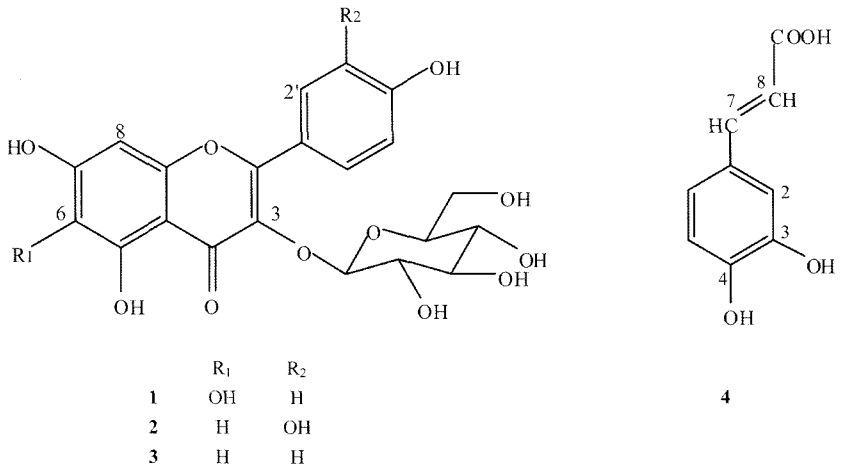
**Abbreviations:** ABTS, 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; TFA, trifluoroacetic acid




| | $R_1$ | $R_2$ |
|---|---|---|
| 1 | OH | H |
| 2 | H | OH |
| 3 | H | H |

4

**Fig. 1. Structures of the isolated flavonol glycosides 1-3 and caffeic acid 4.**

of the ABTS radical at 734 nm [van den Berg *et al.*, 1999; Huang *et al.*, 2005]. The radical-scavenging activities of these compounds and Trolox, the standard reference compound, showed dose-dependent effects on the ABTS radical (Fig. 2). Trolox suppressed the absorbance of the ABTS radical with the $EC_{50}$ value of 14.4 ± 0.22 μM [Yoon *et al.*, 2007], while those of **1-3** were 5.36 ± 0.08, 3.01 ± 0.05, and 4.09 ± 0.02 μM, respectively (Fig. 2), indicating these flavonol glucosides have strong free radical-scavenging activities. Although the $EC_{50}$ values of **1-3** (3.01-5.36 μM) and other flavonol glycosides from the BuOH-soluble fraction of this plant (quercetin 3-*O*-rutinoside, kaempferol 3-*O*-rutinoside and kaempferol 3-*O*-sophoroside; $EC_{50}$ = 5.26 ± 0.10, 6.08 ± 0.03, and 4.07 ± 0.09 μM, respectively [Yoon *et al.*, 2007]) did not show significant differences, quercetin 3-*O*-glucoside **2** had the highest antioxidant activity, suggesting the dihydroxyl group in its B ring could play some role [Re *et al.*, 1999].

**Table 1. $^1$H NMR Spectroscopic Data of Compounds 1, 2, and 3 in $CD_3OD$**

| Proton | 1 | 2 | 3 |
|---|---|---|---|
| 6 | | 6.20 d (2.0) | 6.20 d (2.0) |
| 8 | 6.52 s | 6.39 d (2.0) | 6.39 d (2.0) |
| 2' | 8.04 d (8.8) | 7.70 d (2.0) | 8.05 d (8.8) |
| 3' | 6.88 d (8.8) | | 6.88 d (8.8) |
| 5' | 6.88 d (8.8) | 6.86 d (8.4) | 6.88 d (8.8) |
| 6' | 8.04 d (8.8) | 7.58 dd (8.4, 2.0) | 8.05 d (8.8) |
| G1 | 5.20 d (7.2) | 5.25 d (7.6) | 5.25 d (7.2) |
| G2 | 3.44 m | 3.47 m | 3.42 m |
| G3 | 3.40 m | 3.42 m | 3.40 m |
| G4 | 3.32 m | 3.34 m | 3.27 m |
| G5 | 3.19 m | 3.21 m | 3.19 m |
| G6 | 3.67 dd (12.0, 2.4) | 3.71 dd (12.0, 2.4) | 3.69 dd (12.0, 2.4) |
| | 3.52 dd (12.0, 5.6) | 3.56 dd (12.0, 5.2) | 3.52 dd (12.0, 5.6) |

These values (3.01-6.08 μM) are comparable to that of caffeic acid **4** (5.49 ± 0.04 μM), reported to show a strong free radical-scavenging activity ($EC_{50}$ = 4.6 μM [Lee *et al.*, 2006]).

In conclusion, five flavonol glycosides (6-hydroxy-kaempferol 3-*O*-glucoside, quercetin 3-*O*-rutinoside, quercetin 3-*O*-glucoside, kaempferol 3-*O*-rutinoside, kaempferol 3-*O*-glucoside) and caffeic acid were isolated from the EtOAc-soluble fraction of safflower using reversed phase HPLC (20-40% aqueous acetonitrile

**Table 2. $^{13}$C NMR Spectroscopic Data of Compounds 1, 2, and 3 in $CD_3OD$**

| Carbon | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 158.99 | 158.92 | 158.93 |
| 3 | 135.07 | 135.59 | 135.33 |
| 4 | 179.42 | 179.36 | 179.34 |
| 5 | 147.36 | 162.92 | 162.93 |
| 6 | 130.26 | 99.80 | 99.80 |
| 7 | 154.66 | 165.91 | 165.80 |
| 8 | 94.56 | 94.63 | 94.66 |
| 9 | 151.15 | 158.36 | 158.36 |
| 10 | 105.96 | 105.58 | 105.67 |
| 1' | 122.90 | 122.94 | 122.68 |
| 2' | 132.13 | 117.44 | 132.16 |
| 3' | 115.95 | 145.78 | 115.97 |
| 4' | 161.31 | 149.77 | 161.41 |
| 5' | 115.95 | 115.90 | 115.97 |
| 6' | 132.13 | 123.08 | 132.16 |
| G1 | 104.23 | 104.18 | 103.98 |
| G2 | 75.69 | 75.69 | 75.70 |
| G3 | 78.03 | 78.09 | 78.00 |
| G4 | 71.31 | 71.20 | 71.33 |
| G5 | 78.35 | 78.37 | 78.39 |
| G6 | 62.60 | 62.53 | 62.61 |

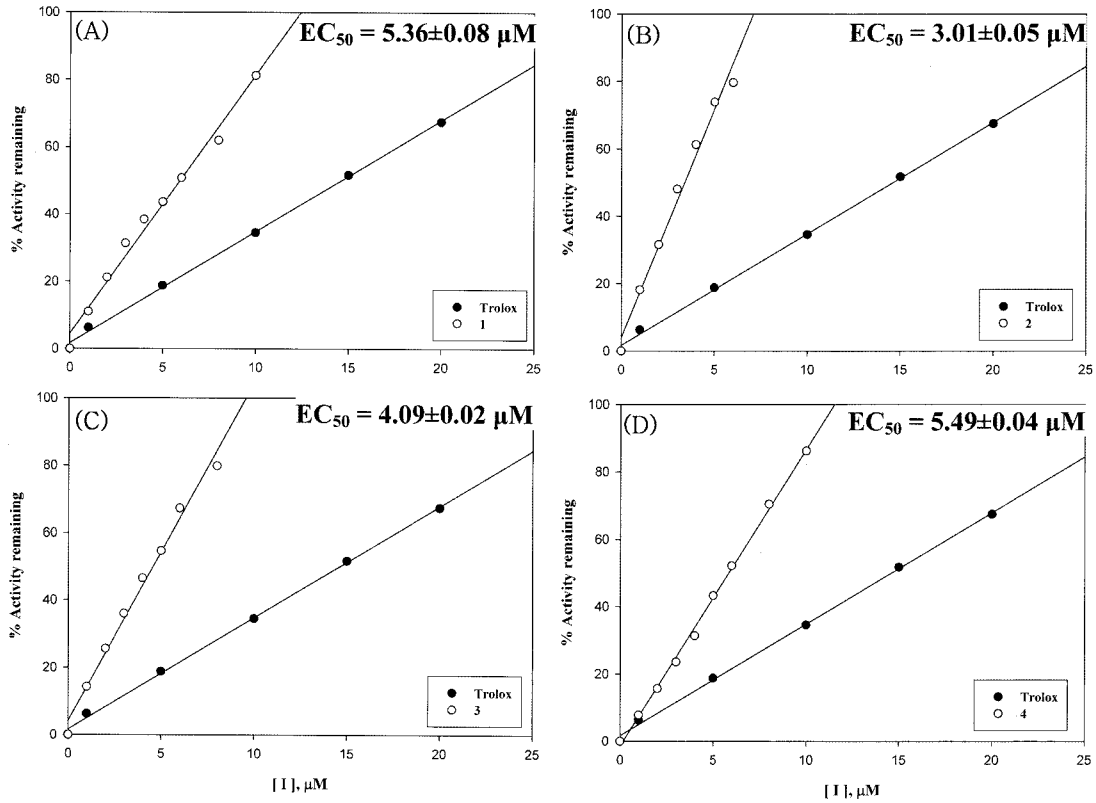



**Fig. 2. The effects of concentration of the antioxidant and Trolox on the inhibition of ABTS.** (A) 6-hydroxykaempferol 3-*O*-glucoside 1, (B) quercetin 3-*O*-glucoside 2, (C) kaempferol 3-*O*-glucoside 3, (D) caffeic acid 4.

gradient, flow rate: 1 mL/min) with the retention times of 24.3, 27.8, 31.3, 31.7, 35.3, and 26.5 min, respectively. The isolated flavonol glycosides, exhibiting similar $EC_{50}$ values with that of caffeic acid **4**, showed strong antioxidant activities against the ABTS radical system.

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## References

Cho MH, Paik YS, and Hahn TR (2000) Enzymatic conversion of precarthamin to carthamin by a purified enzyme from the yellow petals of safflower. *J Agric Food Chem* **48**, 3917-3921.

Flamini G, Antognoli E, and Morelli, I (2001) Two flavonoids and other compounds from the aerial parts of *Centaurea bracteata* from Italy. *Phytochemistry* **57**, 559-564.

Hattori M, Huang XL, Che QM, Kawata Y, Tezuka Y, Kikuchi T, and Namba T (1992) 6-Hydroxykaempferol and its glycosides from *Carthamus tinctorius* petals. *Phytochemistry* **31**, 4001-4004.

Huang D, Ou B, and Prior RL (2005) The chemistry behind antioxidant capacity assays. *J Agric Food Chem* **53**, 1841-1856.

Kazuma K, Takahashi T, Sato K, Takeuchi H, Matsumoto T, and Okuno T (2000) Quinochalcones and flavonoids from fresh florets in different cultivars of *Carthamus tinctorius* L. *Biosc Biotechnol Biochem* **64**, 1588-1599.

Kim JB and Paik YS (1997) Stability of carthamin from *Carthamus tinctorius* in Aqueous solution: pH and temperature effects. *Arch Pharm Res* **20**, 643-646.

Lee JH, Kang NS, Ha TJ, Ko JM, Han WY, Suh DY, Park KH, and Baek IY (2006) Antioxidant activities and determination of phenolic acids from leaves of *Perilla frutescens*. *Agric Chem Biotechnol* **49**, 11-15.

Obara H and Onodera J-i (1979) Structure of carthamin. *Chem Lett* 201-204.

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

van den Berg R, Haenen GRMM, van den Berg H, and Bast A (1999) Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of capacity measurements of mixtures. *Food Chem* **66**, 511-517.

Yin HB and He ZS (2000) A novel semi-quinone chalcone sharing a pyrrole ring *C*-glycoside from *Carthamus tinc-*

*torius*. *Tetrahedron Lett* **41**, 1955-1958.

Yoon HR, Han HG, and Paik YS (2007) Flavonoid glycosides with antioxidant activity from the petals of *Carthamus tinctorius*. *J Appl Biol Chem* **50**, 175-178.

Yoon JM, Cho MH, Park JE, Kim YH, Hahn TR, and Paik YS (2003) Thermal stability of the pigments hydroxysafflor yellow A, safflor yellow B, and precarthamin from safflower (*Carthamus tinctorius*). *J Food Sci* **68**, 839-843.