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홍화의 성분 분리 및 항산화 활성

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Constituents of Flowers of *Carthamus tinctorius* L. and Their Antioxidant Activity

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Abstract – As part of our ongoing study focused on the discovery of antioxidants from natural products by measuring the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity, methanol extract of flowers of *Carthamus tinctorius* L. was found to show potent antioxidant activity. Activity-guided fractionation of the methanol extract lead to the isolation of twenty compounds including two flavonol glycosides, quercertin-3-*O*-β-D-glucopyranoside (**12**) and kaempferol-3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (**18**), two flavanone glycosides, (2*S*)-4',5,6,7-tetrahydroxyflavanone 6-*O*-β-D-glucopyranoside (**15**) and (2*R*)-5,7,8',4-tetrahydroxyflavanone 8-*O*-β-D-glucopyranoside (**16**), and two acetylenic glycosides, 8*Z*-decaene-4,6-diyne-1-*O*-β-D-glucopyranoside (**13**) and 4,6-decadiyne-1-*O*-β-D-glucopyranoside (**14**). Their chemical structures were identified by using spectroscopic analysis. Among them, compounds **12-18** were tested in DPPH assay. Compounds **13-16** were first reported to their antioxidant activity. Quercertin-3-*O*-β-D-glucopyranoside (**12**) showed the most potent inhibitory effect on DPPH with IC_{50} value of 56.7 μM.

Key words–*Carthamus tinctorius*, DPPH, Quercertin-3-*O*-β-D-glucopyranoside, Acetylenic glycoside

홍화(*Carthamus tinctorius* L. Safflower)는 국화과 (Compositae)에 속하는 여러해살이식물로서 원산지는 아프가니스탄의 산악지대 또는 이집트 및 에티오피아라고 알려져 있다.[1)] 꽃이 필 때의 관상화를 그대로 또는 황색색소의 대부분을 제거하고, 압축해서 판상으로 한 것을 약용으로 한다.[2)] 홍람, 홍화, 잇꽃, 잇나물 이라고도 한다. 높이는 1 m 내외이며, 잎은 어긋나고 넓은 바소꼴이며, 톱니끝이 가시처럼 생긴다. 꽃은 7~8월에 피고 엉겅퀴같이 생겼으나 붉은 빛이 도는 노란색이고 가지 끝에 1개씩 달린다. 총포는 잎 같은 포로 싸이고 가장자리에 가시가 있다. 열매는 수과 로서 길이 6 mm이며 윤기가 있고 짧은 관모가 있다. 종자는 흰색이다. 꽃받침 중앙에 1.5~3 cm크기의 관상화가 뭉쳐서 핀다.[3)] 홍화는 본초강목에서 통증을 제거하고, 혈액순환을 원활하게 해준다고 기록되어 있어, 혈행 장해, 통경액, 맹증, 갱년기 장해, 특히 산전 산후의 부인병에 정혈제로 널리 쓰였으며, 한방에서 뇌일혈 후 의 반신불수에 중요하게 쓰인다고 알려져 있다. 또한, 완하 작용 발한 작용, 하혈 작용이 있으며,[4,5)] 기분을 진정시키는 효과가 있다고 알려져 있다. 약리활성에 관한 연구로는 항산화 활성,[6)] 조골모유사세포활성,[7)] 멜라닌 생합성 저해 활성,[8)] 항 바이러스 활성,[9)] 등이 보고 되었다. 홍화로부터 분리 보고된 성분으로는 flavonoid 성분인 kaempferol, quercertin, quercitrin, 6-hydroxy-kaempferol, luteolin, apigenin이 분리 보고 되었고,[10)] quinochalcone계 색소 성분인 safflomin A, safflomin B, hydroxysafflor yellow A, carthamin이 보고되었고,[11)] polyacetylenic glycoside인 4',6'-acetonide-8*Z*-decaene-4,6-diyne-1-*O*-β-D-glucopyranoside, 4,6-decadiyne-1-*O*-β-D-glucopyranoside, acetylenic glucoside, *Z*-decaene-4,6-diyne-1-*O*-β-D-glucopyranoside가 분리 보고 되었다.[12)] 또한 홍화

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에서 분리된 serotonin 유도체의 항산화활성이 보고 되었고,[13] trachorogenin, quercertin, catechin은 tyrosinase를 억제하는 활성이 있다고 보고되었으며, 2-hydroxyarctiin, matairesinol glycoside, sesquiterpene glucoside 등은 항암 효과가 있다고 보고되었다.[14] 최근 보고된 논문에서 페놀성 화합물인 matairesinol 4'-*O*-β-D-glucoside, 8'-hydroxyarctigenin 4'-*O*-β-D-glucoside, matairesinol, 8'-hydroxyarctigenin, *N*-feruloylserotonin 5-*O*-β-D-glucoside, *N*-(p-coumaroyl) serotonin,[13] luteolin 7-*O*-β-D-glucoside, luteolin, acacetin 7-*O*-β-glucuronide, acacetin[10] 등은 항산화 활성을 가진다고 보고되었다.

본 연구에서는 홍화로부터 성분을 분리하고, 분리한 화합물에 대하여 항산화 활성을 측정하였다. Acetylenic glycosides인 8*Z*-decaene-4,6-diyne-1-*O*-β-D-gluco-pyranoside (**13**), 4,6-decadiyne-1-*O*-β-D-glucopyranoside (**14**), flavanone glycoside인 (2S)-4',5,6,7-tetrahydroxyflavanone 6-*O*-β-D-glucopyranoside (**15**), (2*R*)-5,7,8'4-tetrahydroxy flavanone 8-*O*-β-D-glucopyranoside (**16**)의 항산화 활성은 본 연구에서 처음 보고되는 것이다.

재료 및 방법

실험재료 – 홍화는 2008년 3월 대구시 약령시장에서 구입하여 사용하였으며, 표품은 영남대학교 약학대학에 보관하고 있다.

시약 및 기기 – 추출 및 column chromatography용 용매는 시약용 1급을 사용하였다. TLC plate는 Kieselgel 60 F254 (Merck) 및 RP-18 (Whatman)을 사용하였다. Column chromatography용 고정상은 silica gel (70-230 mesh, Merck), Sephadex LH-20 (25-100 μ, Sigma Chem. Co.), MCI-gel CHP-20P (75-150 μ, Mitsubishi Chem. Co.), RP-18 (40-63 μm, Merck), Toyopearl HW-40F (Tosho) 등을 사용하였다.

HPLC의 고정상으로는 Shim-pack PREP-ODS column 20 mm×250 mm (Shimadzu)을 사용하였으며, 이동상의 조성은 MeOH와 H_2O, acetonitrile의 이상 또는 삼상 혼액을 사용하였으며, 시료에 따라서 비율을 정하여 기울기 용리를 하였다.

발색 시약으로는 $FeCl_3$/ethanol 용액, anisaldehyde-sulfuric acid 시액, vanillin-sulfuric acid 시액, phosphomolybdenic acid, Liebermann-Burchard 시액, dragendorff 시액 등을 사용하였다.

HPLC는 LC-10A (Shimadzu)를 사용하였다. Fraction collector는 SF-160 (Advantec)을 사용하였다. EI-MS spectrum은 Micromass spectrum (AUTOSPEC, UK)을 사용하였다. NMR spectrum은 Bruker ARX 250 spectrometer (250 MHz)의 Bruker's standard pulse program을 사용하였으며, 시료는 $CDCl_3$, CD_3OD, pyridine-d_5, 또는 acetone-d_6 (Aldrich Chem. Co.)에 녹여 사용하였고, chemical shift value는 tetramethylsilane (TMS)으로부터 downshift된 part per million (ppm) 단위로 나타내었다.

DPPH free radical 소거법에 의한 항산화활성 측정 – 96 well microplate에 시료 10 μL를 넣고 DPPH solution (2.0×10^{-4}, ethanol) 190 μL를 가한 후 실온에서 30분간 반응시켜 각 반응액의 흡광도를 517 nm에서 측정하였다. 대조군으로는 시료 대신 DMSO를 가해 시료의 흡광도 감소 정도를 조사하였으며, DPPH radical을 50% 소거시키는 시료의 농도를 IC_{50}로 하였다. 각 시료에 대한 DPPH radical 소거작용을 3회 반복하여 측정 하였다.[15,16]

화합물의 분리 및 구조 – 홍화(*Carthamus tinctorius*) 5.5 kg을 MeOH로 3회 추출하여 여과한 후, 감압 농축하여 MeOH추출물(1.5 kg)을 얻었다. 추출물(1.5 kg)을 증류수에 현탁시키고 동량의 *n*-Hexane을 가하여, *n*-Hexane 층과 수층을 분획하는 조작을 3회 반복 실시한 후 감압농축하여 223 g의 *n*-Hexane 분획을 얻었다. 다시 수층에 동량의 EtOAc을 가한 후 3회 반복 분획하여 48 g의 EtOAc분획을 얻었고, 나머지를 물분획 (640 g)으로 하였다. 얻어진 분획을 각종 크로마토그래피법을 통하여 *n*-Hexane 분획에서 화합물 **1-3**을 얻었고, EtOAc 분획에서 화합물 **4-16**을, H_2O 분획에서 화합물 **17-20**을 분리하였다.

β-Amyrin acetate (**1**) – White crystal ; ^{1}H-NMR (250 MHz, $CDCl_3$) ; δ 0.74 (3H, s, H-28), 0.80 (6H, s, C-29 and H-30), 0.93 (6H, s, H-23 and H-24), 1.16 (3H, s, H-25), 1.20-1.97 (s, CH_3, CH_2), 2.02 (3H, s, H-29), 4.47 (1H, dd, *J* = 3.0, 5.0, 12.0, CH-3), 5.15 (1H, m, H-12).^{13}C-NMR (63 MHz, $CDCl_3$) ; δ 14.1 (C-25), 18.2 (C-6), 21.3 (C-32), 22.6 (C-24), 23.4 (C-23), 23.5 (C-11), 23.6 (C-2), 25.9 (C-26), 26.1 (C-27), 26.8 (C-28), 28.0 (C-15), 28.4 (C-12), 31.1 (C-12), 31.6 (C-13), 32.5 (C-20), 32.5 (C-17), 33.3 (C-7), 34.7 (C-21), 36.8 (C-22), 37.1 (C-10), 37.7 (C-4), 38.2 (C-1), 39.7 (C-8), 41.6 (C-14), 46.7 (C-19), 47.2 (C-18), 47.5 (C-9), 55.2 (C-5), 80.9 (C-3), 121.6 (C-12), 145.2 (C-13), 171.05 (C-31).

Trilinolein (**2**) – Yellow oil ; ^{1}H-NMR ($CDCl_3$, 250 MHz) ; δ 0.81-0.85 (9H, m, H-1), 1.57 (6H, m, H-2), 2.01 (12H, t, *J* = 6.1 Hz, H-8, 4), 2.27 (6H, t, *J* = 7.5 Hz, $COCH_2$), 2.73 (6H, t, *J* = 5.6 Hz, H-11), 4.10 (2H, dd, *J* = 11.9, 5.9 Hz,H-α, γ), 4.26 (2H, dd, *J* = 11.9, 4.3 Hz, H-α, γ), 5.21-5.35 (13H, m, β-CH, H-CH_2). ^{13}C-NMR ($CDCl_3$, 63 MHz) ; δ 173.1 (α, γ-CO), 172.7 (β-CO), 130.1 (C-13), 129.9 (C-9), 127.9 (C-10), 127.8 (C-12), 68.7 (C-β), 62.0 (C-α, γ), 33.9 (C-2), 31.4 (C-16), 29.6 (C-7), 29.4 (C-15), 29.2 (C-6), 29.1-29.7 (CH_2), 27.1 (C-8,

C-14), 25.5 (C-11), 24.7 (C-3), 22.6 (C-17), 14.1 (CH_3).

Linoleic acid (**3**) – Yellow oil ; ^{1}H-NMR (250 MHz, CD_3OD) ; δ 0.82-0.85 (3H, m, H-1), 1.28 (2H, m, H-2), 1.32 (2H, m, H-3), 1.57 (2H, m, H-5), 2.03 (6H, q, *J* = 6.3, H-8, 14), 2.30 (2H, t, *J* = 7.5, H-17), 2.74 (H, t, *J* = 6.3, H-CH_2), 5.23-5.31 (4H, m, H-5,6,8,9). ^{13}C-NMR (63 MHz, CD_3OD) ; δ 13.4 (CH_3). 22.65 (C-17), 24.9 (C-3), 25.6(C-11), 27.2 (C-8 and C-14), 29.1-29.7 (CH_2), 29.2 (C-6), 29.3 (C-15), 29.6 (C-7), 31.5 (C-16), 34.0 (C-2), 128.0 (C-12), 128.0 (C-10), 129.8 (C-9), 129.8 (C-13), 176.8 (C-1).

Quercertin (**4**) – Yellow powder ; ^{1}H-NMR (250 MHz, CD_3OD) ; δ 6.18 (1H, d, *J* = 2.02, H-6), 6.38 (1H, d, *J* = 2.03, H-7), 6.89 (1H, d, *J* = 8.5,H-2'), 7.63 (1H, dd, *J* = 2.1, 8.51, H-5'), 7.72 (1H, d, *J* = 2.02, H-6'). ^{13}C-NMR (63 MHz, CD_3OD) ; δ 151.4 (C-2), 137.6 (C-3), 177.5 (C-4), 163.8 (C-5), 99.2 (C-6), 165.6 (C-7), 94.4 (C-8), 158.2 (C-9), 105.8 (C-10), 124.1 (C-1'), 115.9 (C-2'), 146.2 (C-3'), 150.4 (C-4'), 116.2 (C-5'), 121.7 (C-6').

Apigenin (**5**) – Yellow powder ; ^{1}H-NMR (250 MHz, CD_3OD) ; δ 6.19 (1H, d, *J* = 2.05, H-6), 6.44 (1H, d, *J* = 2.12, H-8), 6.58 (1H, s, H-3), 6.92 (2H, d, *J* = 8.92, H-3' and H-5'), 7.85 (2H, d, *J* = 8.95, H-2' and H-6'). ^{13}C-NMR (63 MHz, CD_3OD) ; δ 164.3 (C-2), 103.6 (C-3), 182.6 (C-4), 162.9 (C-5), 99.8 (C-6), 165.7 (C-7), 94.7 (C-8), 162.5 (C-9), 104.7 (C-10), 128.7 (C-1'), 135.8 (C-2', C-6'), 116.7 (C-3', C-5'), 158.3 (C-4').

Kaempferol (**6**) – Yellow powder ; ^{1}H-NMR (250 MHz, CD_3OD) ; δ 6.06 (1H, d, *J* = 2.07, H-6), 6.27 (1H, d, *J* = 2.07, H-8), 6.78 (2H, d, *J* = 9.80, H-3' and H-5'), 7.97 (1H, d, *J* = 4.95, H-2' and H-6'). ^{13}C-NMR (63 MHz, CD_3OD) ; δ 147.9 (C-2), 137.2 (C-3), 177.3 (C-4), 162.5 (C-5), 99.2 (C-6), 165.6 (C-7), 94.4 (C-8), 160.5 (C-9), 104.5 (C-10), 123.7 (C-1'), 130.7 (C-2' and C-6'), 116.3 (C-3' and C-5'), 158.2 (C-4').

Luteolin (**7**) – Yellow powder ; ^{1}H-NMR (250 MHz, CD_3OD) ; δ 6.15 (1H, d, *J* = 1.97, H-6), 6.39 (1H, d, *J* = 2.02, H-8), 6.49 (1H, s, H-2'), 6.84 (1H, d, *J* = 8.85, H-3), 7.34 (1H, m, H-5' and H-6'). ^{13}C-NMR (63 MHz, Pyridine-d_5) ; δ 164.7 (C-2), 104.9 (C-3), 182.6 (C-4), 163.7 (C-5), 99.8 (C-6), 165.7 (C-7), 94.7 (C-8), 158.4 (C-9), 103.9 (C-10), 122.8 (C-1'), 114.5 (C-2'), 147.6 (C-3'), 151.5 (C-4'), 116.7 (C-5'), 119.4 (C-6').

Ferulic acid (**8**) – Yellow powder ; ^{1}H-NMR (250 MHz, CD_3OD) ; δ 3.82 (3H, s, H-10), 6.24 (1H, d, *J* = 15.9, H-8), 6.73 (1H, d, *J* = 8.15, H-3), 6,97 (1H, dd, *J* = 1.9, 8.2, H-6), 7.11 (1H, d, *J* = 1.75, H-2), 7.51 (1H, d, *J* = 15.9, H-7). ^{13}C-NMR (63 MHz, CD_3OD) ; δ 126.8 (C-1), 123.0 (C-2), 110.5 (C-3), 149.4 (C-4), 148.4 (C-5), 115.2 (C-6), 115.4 (C-7), 145.7 (C-8), 170.2 (C-9), 55.4 (C-10).

Syringaresinol (**9**) – Yellow gum ; ^{1}H-NMR (600 MHz, $CDCl_3$) ; δ 3.07 (2H, m, H-1 and H-5), 3.87 (12H, s, H-3', 5', 3" and 5"), 3.89 (2H, d, *J* = 3.6, H-2 and H-6), 4.26 (2H, dd, *J* = 6.6, 9.2, H-2 and H-6), 4.70 (2H, d, *J* = 3.6, H-4 and H-8), 5.49 (OH, s, OH-4' and OH-4"), 6.56 (4H, s, H-2', 6', 2" and 6"). ^{13}C-NMR (150 MHz, $CDCl_3$); δ 54.3 (C-1 and C-5), 71.8 (C-2 and C-6), 86.1 (C-4 and C-8), 56.4 (C-3', 5', 3" and 5"), 132.1 (C-1' and C-1"), 102.7 (C-2', 6', 2" and 6"), 147.1 (C-3', 5', 3" and 5"), 134.3 (C-4' and C-4").

Isoferulic acid (**10**) – Yellow powder ; ^{1}H-NMR (250 MHz, CD_3OD) ; δ 3.83 (3H, s, H-10), 6.21 (1H, d, *J* = 15.9, H-8), 6.88 (1H, d, *J* = 8.0, H-3), 6.98 (2H, d, *J* = 8.2, H-6 and H-2), 7.49 (1H, d, *J* = 1.6), 7.51 (1H, d, *J* = 15.9, H-7). ^{13}C-NMR (63 MHz, CD_3OD) ; δ 127.9 (C-1), 121.7 (C-2), 111.4 (C-3), 150.4 (C-4), 147.1 (C-5), 113.6 (C-6), 115.4 (C-7), 145.6 (C-8), 169.8 (C-9), 55.3 (C-10).

4-Hydroxybenzoic acid (**11**) – Yellow powder ; ^{1}H-NMR (250 MHz, CD_3OD) ; δ 6.73 (2H, d, *J* = 7.4, H-5 and H-3), 7.78 (2H, d, *J* = 7.4, H-6 and H-2).^{13}C-NMR (63 MHz, CD_3OD) ; δ 122.7 (C-1), 133.0 (C-2 and C-6), 116.0 (C-3 and C-5), 163.4 (C-4), 170.1 (C-7).

Quercertin-3-*O*-β-D-glucopyranoside (**12**) – Yellow powder ; ^{1}H-NMR (250 MHz, CD_3OD) ; δ 5.20 (1H, d, *J* = 7.42, H-1"), 6.14 (1H, d, *J* = 2.02, H-6), 6.32 (1H, d, *J* = 2.03, H-7), 6.81 (1H, d, *J* =8.5, H-2'), 7.53 (1H, dd, *J* = 2.1, 8.51, H-5'), 7.65 (1H, d, *J* = 2.02, H-6'). ^{13}C-NMR (63 MHz, CD_3OD) ; δ 151.4 (C-2), 137.6 (C-3), 178.5 (C-4), 162.0 (C-5), 98.9 (C-6), 165.1 (C-7), 93.7 (C-8), 158.0 (C-9), 104.7 (C-10), 122.2 (C-1'), 115.0 (C-2'), 144.9 (C-3'), 148.8 (C-4'), 115.0 (C-5'), 116.5 (C-6'), 104.7 (C-1"), 74.7 (C-2"), 77.1 (C-3"), 70.2 (C-4"), 77.4 (C-5"), 61.1 (C-6").

8Z-Decaene-4,6-diyne-1-*O*-β-D-glucopyranoside (**13**) – Yellow syrup ; ^{1}H-NMR (CD_3OD, 250 MHz) ; δ 1.90 (2H, m, CH_3-10, H-7), 2.52 (2H, t, *J* = 7.0 Hz, H-3), 4.30 (1H, d, *J* = 7.80 Hz,H-1'). ^{13}C-NMR (CD_3OD, 63 MHz) ; δ 69.2 (C-1), 29.5 (C-2), 16.5 (C-3), 85.1 (C-4), 66.0 (C-5), 79.3 (C-6), 72.5 (C-7), 109.8 (C-8), 143.2 (C-9), 16.7 (C-10), 104.1 (C-1'), 74.7 (C-2'), 77.6 (C-3'), 71.1 (C-4'), 77.5 (C-5'), 62.3 (C-6').

4,6-Decadiyne-1-*O*-β-D-glucopyranoside (**14**) – Bright

yellowish syrup ; ^{1}H-NMR (CD_3OD, 250MHz) ; δ 0.78 (3H, dt, *J* = 2.2, 7.5, H-10), 1.47 (2H, m, H-9), 1.75 (2H, m, H-2), 2.08 (2H, t, *J* = 6.7Hz, H-3), 2.42 (2H, t, *J* =7.0, CH_2 -3), 4.30 (1H, d, *J* =7.80 CH-1'). ^{13}C-NMR (CD_3OD, 63 MHz) ; δ 67.8 (C-1), 28.8 (C-2), 15.7 (C-3), 77.8 (C-4), 67.8 (C-5), 66.2 (C-6), 78.1 (C-7), 20.7 (C-8), 21.7 (C-9), 13.1 (C-10), 104.2 (C-1'), 74.8 (C-2'), 77.4 (C-3'), 71.1 (C-4'), 77.4 (C-5'), 62.3 (C-6').

(2*S*)-4',5,6,7-Tetrahydroxyflavanone 6-*O*-β-D-glucoside (**15**) – Yellowish powder ; ^{1}H-NMR (DMSO-d_6, 250 MHz) ; δ 5.43 (1H, dd, *J* = 12.5, 3.0Hz H-3',5'), 3.28 (1H, dd, *J* = 17.0, 12.5 Hz H-3), 2.68 (1H, dd, *J* = 17.0, 3.0 Hz), 7.31 (2H, d, *J* = 8.5 Hz,), 6.79 (2H, t, *J* = 8.5 Hz, H-8), 12.24 (1H, s, 5-OH), 0.92 (3H, t, *J* = 7.2 Hz, H-10), 9.60 (3H, d, *J* = 7.8 Hz, OH-4'). ^{13}C-NMR (DMSO-d_6, 63 MHz) ; δ 197.1 (C-4), 159.4 (C-7), 15.6 (C-3), 158.6 (C-4), 157.7(C-5), 155.0 (C-6), 128.8 (C-7), 128.4 (C-8), 126.2 (C-9), 115.2 (C-10), 104.7 (C-1'), 101.8 (C-2'), 95.0 (C-3'), 78.5 (C-4'), 77.2 (C-5'), 76.1 (C-5').

(2*R*)-5,7,8',4-Tetrahydroxy flavanone 8-*O*-β-D-glucoside (**16**) – Yellow powder ; ^{1}H-NMR (DMSO-d_6, 250 MHz) ; δ 5.43 (1H, d, *J* = 11.8, 3.0Hz H-3',5'), 3.28 (1H, dd, *J* = 17.2, 11.8 Hz, H-3), 2.75 (1H, dd, *J* = 17.0, 3.0 Hz), 7.37 (2H, d, *J* = 8.5 Hz, H-2',6'), 6.79 (2H, t, *J* = 8.5 Hz, H-8), 4.61 (2H, d, *J* = 6.4 Hz, H-2''), 3.70 (3H, m, H-6'). ^{13}C-NMR (DMSO-d_6, 62.9 MHz) ; δ 159.4 (C-7), 15.6

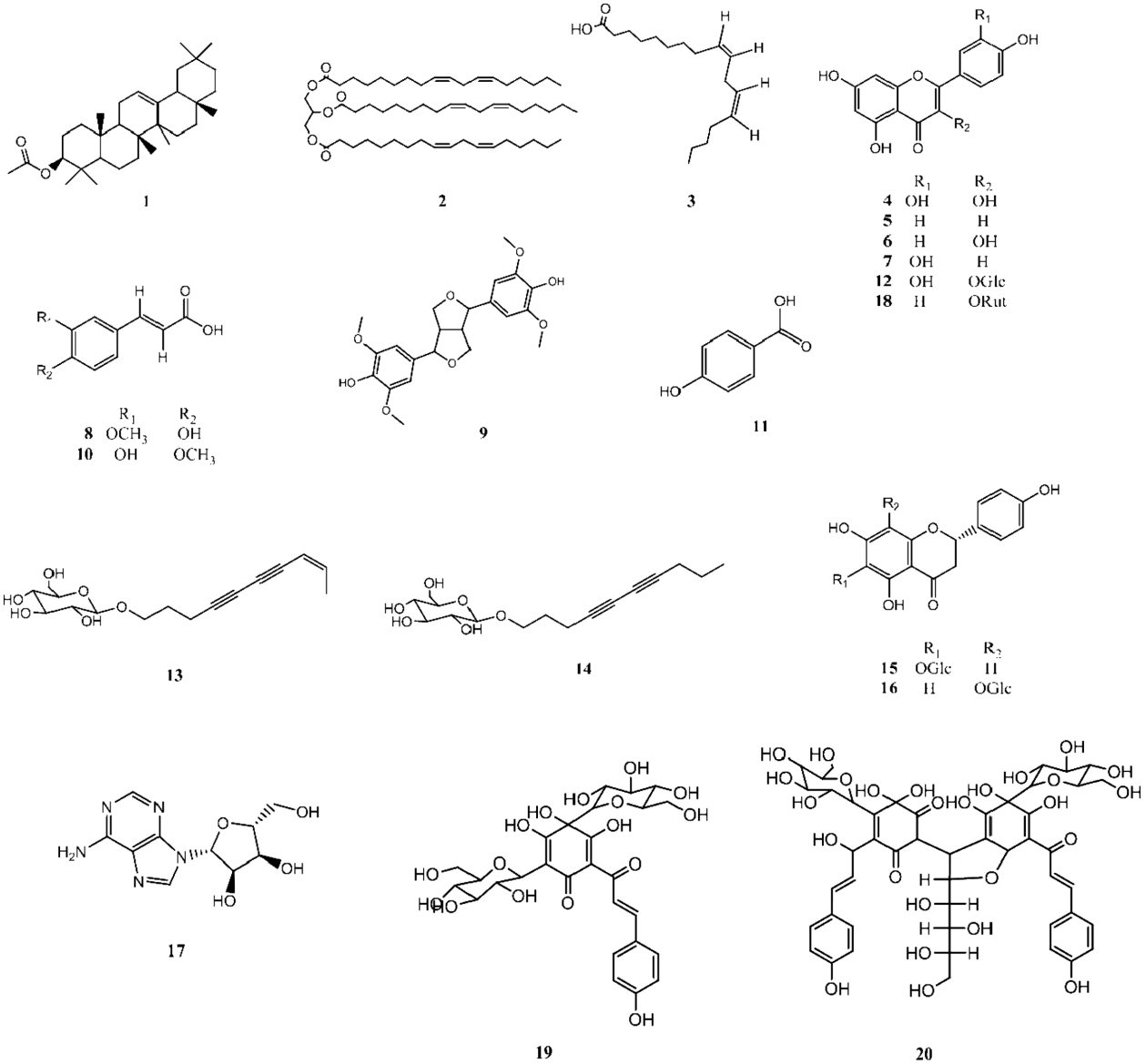

Fig. 1. Chemical structures of constituents isolated from *C. tinctorius.*

(C-3), 158.6 (C-4), 157.7(C-5), 155.0 (C-6), 128.8 (C-7), 128.4 (C-8), 126.2 (C-9), 115.2 (C-10), 155.5 (C-4'), 107.2 (C-1"), 101.8 (C-2"), 95.0 (C-3"), 80.61 (C-4"), 77.2 (C-5"), 62.2 (C-6").

Adenosine (**17**) – Yellow crystal ; ^{1}H NMR (DMSO-d_6-250 MHz) ; δ 3.59 (2H, dt, *J* = 3.6, 12.3 Hz, H-5'), 3.97 (1H, q, *J* = 3.3 Hz, H-4'), 4.13 (1H, dd, *J* = 3.1, 5.0 Hz, H-3), 4.57 (1H, dd, *J* = 5.2, 5.7 Hz, H-2'), 5.85 (1H, d, *J* = 6.24, H-1'), 8.13 (1H, s, H-2), 8.32 (1H, s, H-8). ^{13}C-NMR (DMSO-d_6, 63 MHz) ; δ 152.3 (C-2), 149.0 (C-4), 119.3 (C-5), 156.1 (C-6), 138.8 (C-8), 87.9 (C-1'), 73.4 (C-2'), 70.6 (C-3'), 85.8 (C-4'), 61.6 (C-5').

Kaempferol 3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (**18**) – Yellow powder ; ^{1}H-NMR (DMSO-d_6, 250 MHz) ; δ 6.06 (1H, d, *J* = 2.07, H-6), 6.27 (1H, d, *J* = 2.07, H-8), 6.78 (2H, d, *J* = 9.80, H-3' and H-5'), 7.97 (1H, d, *J* = 4.95, H-2' and H-6') 5.12 (1H, d, *J* = 7.0, H-1"), 1.12 (3H, d, *J* = 6.0, H-6"'). ^{13}C-NMR (DMSO-d_6, 63 MHz) ; δ 147.9 (C-2), 137.2 (C-3), 177.3 (C-4), 162.5 (C-5), 99.2 (C-6), 165.6 (C-7), 94.4 (C-8), 160.5 (C-9), 104.5 (C-10), 123.7 (C-1'), 130.7 (C-2' and C-6'), 116.3 (C-3' and C-5'), 158.2 (C-4') (C-1"), 75.7 (C-2"), 77.1 (C-3"), 71.3 (C-4"), 77.1 (C-5"), 68.7 (C-6") 102.3 (C-1"'), 72.2 (C-2"'), 73.8 (C-3"'), 71.3 (C-4"'), 69.7 (C-5"'), 17.9 (C-6"').

Hydroxysafflor yellow A (**19**) – Yellow powder ; ^{1}H-NMR (CD_3OD, 250MHz) ; δ 7.24 (d, *J* = 15.6 Hz, H-8), 7.42 (d, *J* = 15.6 Hz, H-9), 7.43 (d, *J* = 7.1 Hz, H-11,15), 6.80 (d, *J* = 7.1 Hz, H-12, 14), 3.85 (d, *J* = 9.5 Hz, H-G1), 3.46 (t, *J* = 9.3 Hz, H-G2), 3.28 (t, *J* = 9.0 Hz, H-G3), 3.15 (m, H-G4), 3.50 (m, H-G5), 3.70 (m, H-G6), 4.40 (d, *J* = 10.0 Hz, H-G1"), 4.07 (t, *J* = 9.5 Hz, H-G2"), 3.36 (t, *J* = 8.8 Hz, H-G3"), 3.15 (m, H-G4"), 3.32 md, H-G5"), 3.43 (dd, *J* = 12.7, 4.4 Hz, d, *J* = 9.5 Hz, H-G6"). ^{13}C-NMR (CD_3OD, 63MHz) ; δ 189.3 (C-1), 96.9 (C-2), 181.1 (C-3), 83.2 (C-4), 194.8 (C-5), 103.1 (C-6), 178.3 (C-7), 118.3 (C-8), 138.1 (C-9), 125.1 (C-10), 127.4 (C-11, 15), 113.3 (C-12, 14), 154.8 (C-13), 82.9 (C-G1), 66.8 (C-G2), 75.0 (C-G3), 76.6 (C-G4), 66.83 (C-G5), 57.90 (C-G6), 71.5 (C-G1"), 66.0 (C-G2"), 75.8 (C-G3"), 66.6 (C-G4"), 77.2 (C-G5"), 58.2 (C-G6").

Safflomin B (**20**) – Yellow powder ; ^{1}H-NMR (CD_3OD, 250 MHz) ; δ 7.45 (d, *J* = 15.8 Hz, H-8), 7.71 (d, *J* = 15.8 Hz, H-9), 7.53 (d, *J* = 8.5 Hz, H-11,15), 3.56 (m, H-G1), 3.32 (m, H-G2), 3.24 (m, H-G3), 3.24 (m, H-G4), 3.68 (m, H-G5), 3.86 (d, *J* = 12.2 Hz, H-G6), 3.58 (m, H-G6), 7.55 (d, *J* = 15.8 Hz, H-8'), 7.74 (d, *J* = 15.8 Hz, H-9'), 7.50 (d, *J* = 8.5 Hz, H-11',15'), 6.80 (d, *J* = 8.5 Hz, H-12',14'), 3.68 (m, H-G1'), 3.27 (m, H-G2'), 3.42 (m, H-G3'), 2.28 (d, *J* = 9.58 Hz, H-G4'), 3.41 (m, H-G5'), 3.63 (m, H-G6'), 3.38 (m, H-G6'), 4.94 (d, *J* = 8.0 Hz, H-G1"), 5.01 (t, *J* = 6.8 Hz, H-G2"), 3.97 (d, *J* = 5.8 Hz, H-G3"), 3.67 (m, H-G4"), 3.79 (dd, *J* = 11.0, 2.7 Hz, H-G5"), 3.59 (m, H-G6"). ^{13}C-NMR (CD_3OD, 63 MHz) ; δ 191.0 (C-1), 109.2 (C-2), 175.6 (C-3), 82.2 (C-4), 196.0 (C-5), 113.6 (C-6), 180.9 (C-7), 119.3 (C-8), 144.3 (C-9), 128.3 (C-10), 131.8 (C-11, 15), 116.9 (C-12, 14), 161.5 (C-13), 88.4 (C-G1), 79.8 (C-G2), 71.2 (C-G3), 82.2 (C-G4), 72.2 (C-G5), 62.7 (C-G6), 86.2 (C-G1'), 79.5 (C-G1'), 68.9 (C-G1'), 80.4 (C-G1'), 70.2 (C-G1'), 60.7 (C-G1'), 37.4 (C-G1") ,94.9 (C-G2"), 72.8 (C-G3"), 70.9 (C-G4"), 72.9 (C-G5"), 65.0 (C-G6").

결과 및 고찰

홍화의 함유 성분을 분리하고 얻어진 화합물에 대하여, DPPH radical 소거효능을 측정하였다. 분리된 화합물은 각종 spectral data를 검토하여 화합물의 구조를 추정하고 해당하는 화합물의 spectral data를 문헌에 소개된 것과 대조하여 각각 β-amyrin acetate (**1**),[17] trilinolein (**2**),[18] linoleic acid (**3**),[18] quercertin (**4**),[10] apigenin (**5**),[12] kaempferol (**6**),[10] luteolin (**7**),[10] ferulic acid (**8**),[19] syringaresinol (**9**), isoferulic acid (**10**),[19] 4-hydroxybenzoic acid (**11**),[19] quercertin-3-*O*-β-D-glucopyranoside (**12**),[10] 8*Z*-decaene-4,6-diyne-1-*O*-β-D-glucopyranoside (**13**),[12] 4,6-decadiyne-1-*O*-β-D-glucopyranoside (**14**),[12] (2*S*)-4',5,6,7-tetrahydroxyflavanone 6-*O*-β-D-glucoside (**15**),[20] (2*R*)-5,7,8',4-tetrahydroxy flavanone 8-*O*-β-D-glucoside (**16**),[21] adenosine (**17**), kaempferol 3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (**18**),[12] hydroxysafflor yellow A (**19**)[11], safflomin A (**20**)[11]로 동정하였다. 분리한 화합물 중 항산화활성이 이미 보고된 화합물을 제외한 화합물 **13-17**의 DPPH radical 소거능에 의한 항산화활성을 측정하였고, 이를 Table I에 나타내었다. Acetylenic glycoside인 화합물 **13**, **14**는 활성을 보이지 않았다. Flavanone glycoside인 화합물 **15**, **16**의 IC_{50}는 각각 63.1, 68.8 μM이었다. 화합물 **12**의 IC_{50}는 56.7 μM로서 화합물 **15**, **16** 와 거의 동등한 radical 소거 활성을 나타냈다. 이와 같은 결과는 활성에 중요한 영향을 미치는 것이 hydroxyl group의 수나 위치에 있을 것이라는 보고에 따라[22], 화합물 **15**, **16**의 분자구조에서 당의 위치만 다를 뿐 같은 골격을 가지고 있기 때문에 이러한 동일한 골격에 결합된 당의 위치는 radical 소거능에 큰 영향을 미치지 않기 때문인 것으로 판단된다. 이상의 결과로 볼 때, 홍화에서 분리한 화합

Table I. DPPH Radical scavenging effects of constituents isolated from *C. tinctorius*

Compound	IC_{50} μM
12	56.7
13	-
14	-
15	63.1
16	68.8
17	-
18	-
Quercertin[b)]	20.3

[a)]The values indicate 50% decrease of DPPH radical and are the means of triplicate data.
[b)]Positive control.

물 **12-18**은 홍화의 항산화활성을 뒷받침하는 하나의 근거로 사료된다.

결 론

홍화의 MeOH엑스로부터 20종의 화합물을 분리하여 각각 β-amyrin acetate (**1**), trilinolein (**2**), linoleic acid (**3**), quercertin (**4**), apigenin (**5**), kaempferol (**6**), luteolin (**7**), ferulic acid (**8**), syringaresinol (**9**), isoferulic acid (**10**), 4-hydroxybenzoic acid (**11**), quercertin-3-*O*-β-D-glucopyranoside (**12**), 8*Z*-decaene-4,6-diyne-1-*O*-β-D-gluco-pyranoside (**13**), 4,6-decadiyne-1-*O*-β-D-glucopyranoside(**14**), (2*S*)-4',5,6,7-tetrahydroxyflavanone 6-*O*-β-D-glucopyranoside (**15**), (2*R*)-5,7,8',4-tetrahydroxyflavanone 8-*O*-β-D-glucopyranoside (**16**), adenosine (**17**), kaempferol 3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (**18**), hydroxysafflor yellow A (**19**), safflomin A (**20**)로 구조를 동정하였다. 그 중 화합물 **12-18**의 항산화활성을 DPPH radical 소거능을 이용하여 측정하였고, **13-16**의 항산화활성은 처음 보고되는 것이다. Quercertin-3-*O*-β-D-glucopyranoside (**12**)가 가장강한 활성을 보여주었으며, IC_{50} value는 56.7 μM이었다.

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