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Antioxidant activity of compounds isolated from *Salvia plebeia*

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Abstract

Six compounds, hispidulin-glucuronide (**1**), hispidulin-7-*O*-D-glucoside (**2**), 6-methoxy-luteolin-7-glucoside (**3**), β-sitosterol (**4**), 2′-hydroxy-5′-methoxybiochanin A (**5**) and coniferyl aldehyde (**6**), were isolated from *Salvia plebeia* and identified by UV, IR, Mass, ^{1}H and ^{13}CNMR spectra. Their antioxidant activities were investigated individually and compared with butylatedhydroxytoluene (BHT) (**8**) and α-tocopherol (**7**) by the oxidative stability instrument (OSI) at 100°C. Compounds **3**, **4** and **5** had strong antioxidant activities, but compounds **1**, **2** and **6** had low antioxidant activities at 0.02 and 0.04% levels.

Keywords: *Salvia plebeia*; Natural antioxidant; Antioxidant activity; Compound

1. Introduction

Since people are particularly concerned about the quality and the safety of their foods, only selected food additives are added for protection them from off-flavour. Antioxidants are often used in oils and fatty foods to retard their autoxidation. The synthetic antioxidants, previously widely used, are now doubted toxicologically (Grice, 1986; Wichi, 1988). So scientist have focussed on natural antioxidants (Chipault et al., 1952; Su et al., 1986). Chinese medicine, herbs and spices are a major source of natural antioxidants.

Many Chinese traditional medicines are plants, and 700 species of herbs were screened for natural antioxidants. Among them, 64 were found to possess obvious antioxidant activity, and 24 showed strong antioxidant activity (Weng, Cao, Dong & Duan, 1998; Weng, Ren, Duan, Dong & Jiang, 1998). One of these herbs is *Salvia plebeia*. It is a small herb which grows indigenously in wet places almost throughout China and India, belonging to the *Labiateae*. This plant is valued for its medicinal properties, and in Chinese traditional medicine is named Lizhicao (Jian, Lou & Zheng, 1987). Previous phytochemical studies of this plant reported that it contained flavones (Gupta, Ayengar & Rangaswami, 1975), lignans (Plattner & Powell, 1978; Richard & Ronald, 1976) and diterpenoids (Maria, Mashooda, Antonio, Forancisco & Benjamin, 1986). Its powder exhibited strong antioxidant activity (Weng et al., 1998) and three compounds had weak antioxidant activity (Weng et al.,1997). So there must be some compounds possesing very strong antioxidants properties occuring in *Salvia plebeia*, but these compounds have got to be isolated from the plant. We therefore assume that there must be other compounds occurring in *Salvia plebeia* which possess very strong antioxidant properties, which have not yet been isolated from the plant.

This paper reports the investigation of antioxidant activities of six compounds isolated from this plant by chromatographing on a silica gel column. Their antioxidant activities were determined by the oxidative stability instrument (OSI) in lard at l00°C.

2. Materials and methods

2.1. Materials

The *S. plebeia* was collected at Yantai, Shandong Province, PR China in 1997, dried with ventilation at ambient temperature, and stored at 4°C until use.

2.2. Chemicals

BHT and α-tocopherol, food grade antioxidants, were purchased from a Chemical Company, Guangzhou,

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China. Silica gel was obtained from Qingdao Ocean Chemical Factory, China. Lard was rendered in the laboratory from fresh pig fat tissue, purchased from Yantai Slaughter House, China. $FeCl_36H_20$, C.P., brown crystalline was purchased from Jinshan Chemical Factory, China.

2.3. Extraction

One kilogram of air-dried and powdered herb of *S. plebeia* was exhaustively extracted with methanol at room temperature. The extract was concentrated under reduced pressure. The dried extract was suspended in H_2O, and extracted with petroleum, chloroform, ethyl acetate and butanol, respectively.

2.4. Chromatographing on silica gel column

Twelve grams of petroleum extract was obtained and chromatographed on a silica gel column (200–300 mesh, 120 g) with a petroleum/EtOAc as the developing solvent. A combination of appropriate fractions (monitored by TLC analysis) led to three fractions. From fraction 1 (petroleum/EtOAc 20:1) a crude crystalline material was obtained and purified by rechromatographing on a silica gel column (300–400, mesh) with petroleum/acetone (10:1), to give conferyl aldehyde (compound **6**, 30 mg) and β-sitosterol (compound **4**, 40 mg).

Nine grams of $CHCl_3$ extract were subjected to column chromatography (CC) on a silica gel column (200–300 mesh, 100 g) using petroleum/EtOAc gradient as developing solvent to give five fractions according to TLC analysis. The 2′-hydroxy-5′-methoxybiochanin A (compound **5**, 40 mg) was obtained from fraction 2 after repeated CC on silica gel column (300–400 mesh, benzene/acetone 15:1) and purified by preparing TLC followed by recrystallization.

Fourteen grams of EtOAc extract was chromatographed on a silica gel column (200–300 mesh, 150 g) with $CHCl_3$/MeOH to give five fractions. Fractions 2 and 3 were repeatedly chromatographed on silica gel (300–400 mesh, $CHCl_3$/acetone 15:1,10:1) to give hispidulin-7-*O*-D-glucoside (compound **2**, 50 mg) and 6-methoxyluteolin-7-glycoside (compound **3**, 55 mg).

The BuOH extract (12 g) was applied to a silica gel column (200–300 mesh, 120 g) and eluted from $CHCl_3$/MeOH to give three fractions. Fraction 1 was chromatographed on silica gel (300–400 mesh, $CHCl_3$/MeOH 10:1) and yielded hispidulin-7-glucuronide (compound **1**, 30 mg).

2.5. Acid hydrolysis

Compounds **1**, **2** and **3** (20 mg each) were added to 15 ml of 1 M HCl–MeOH solution and refluxed for 10 h. The MeOH was then evaporated under reduced pressure and the residue was added to water and extracted with EtOAc. The aqueous layer was neutralized with $NaHCO_3$ and evaporated to dryness. The residue were identified as D-glucose and D-glucuronide by paper chromatography, using EtOAc/Pyridine/H_2O (12:5:4) as developing solvent. The sugars were further confirmed by GC (trimethylsilyl derivatives) and showed the same R_f as those of the derivatives of D-glucose and D-glucuronide. The EtOAc solution was evaporated to dryness and the residue was chromatographed on silica gel by gradient elution with $CHCl_3$/MeOH. From the elution with $CHCl_3$/MeOH (10:1), the aglycones of compounds **1**, **2** and **3** were 10, 8 and 10 mg.

2.6. Recording spectra

MPs were determined by Kofler-microscope (Reichert) uncorr, optical rotation by Polarimeter 241 (Perkin Elmer), solvent MeOH. IR-spectra were recorded on a Nicolet 5DX IR spectrometer. ^1H and ^{13}CNMR spectra were recorded with a Bruker AM-400, solvent pyridine-d_5, using TMS as internal standard. ElMS and FAB-MS were determined with a MS 50 (A.E.I. Brumer) and a ZAB-HS mass spectrometer.

2.7. Antioxidant activity

Antioxidant activities were studied in lard with an Omnion OSI, MA, USA, at 100°C. The airflow rate was fixed at 20 l/h. BHT and α-tocopherol were used as comparison samples.

The protection factors (Pf) = the induction period (IP) of lard with antioxidant/the IP of lard without antioxidant. The IP results were duplicates.

3. Results and discussion

Compound **1** was isolated and recrystallized from $CHCl_3$/MeOH (10:1) as yellow needles. It was determined as $C_{22}H_{20}O_{12}$ by FAB-MS data (m/z 499$[M+Na]^+$, 483$[M+Li]^+$) and by counting carbons and hydrogens from its ^{13}C NMR DEPT. The IR spectra showed sugar at 3300, 1050 cm^{-1}. The UV, IR, MP, ^1H and ^{13}C NMR data of aglycone were identified as hispidulin (Jian et al., 1987), and the sugar was identified as glucuronide. So compound **1** was identified as hispidulin-7-glucuronide. IR (KBr, cm^1): 3300(OH), 1692 (C=O), 1600, 1581, 1497(C=C), 1050(glc). The detailed spectral data of glucuronide are listed in Table 1.

Compound **2** was obtained from $CHCl_3$/acetone (15:1) as yellow needles. Its molecular formula is $C_{22}H_{22}O_{11}$ based on FAB-MS data (m/z 485 $M+Na]^+$ 469$[M+Li]^+$) and by counting carbons and hydrogens from the data of its ^1H and ^{13}C NMR DEPT spectra.

Table 1
Spectral and melting point data of the compounds isolated from *S. plebeia*

Compound	^{1}H NMR (400 hz) (in prydin-d_5, TMS as int. standard)	^{13}C-NMR (100 hz) (in prydine-d_5, TMS as int. standard)	MS (m/z)	IR (cm^1) (KBr)	UV (nm) (MeOH)	MP·C
1	2.90(t,1H),3.06(m,2H),3.10(t,1H), 3.75(S,3H),4.08(d,1H),4.41 (d,1H),6.87(d,2H),7.41(d,1H)	55.8,71.8,71.9,75.1,78.4, 78.6,93.9,104.9(2C),106.9, 115.3,117.5,121.1(2C),143.3, 146.7,152.7,153.3,153.7, 154.2,157.5, 180.5	499[M + Na] 483[M + Li]	3300,1692,1600, 1581,1497,1050	275,233	255–257
2	2.93(m,2H),3.14(m,2H),3.63 (brd,1H),3.69(brd, 1H),3.71 (S,3H),4.42(d, 1H), 6.53(S,1H), 6.64(S,1H),6.85(d,2H),7.40 (d,1H)	55.9,61.3,70.2,72.8,75.8, 77.1,94.1,102.9,103.6,105.2, 114.5,118.4,121.9,122.1, 142.8,147.1,153.5,154.2, 156.0,156.9,158.3, 181.6	485[M + Na] 469[M + Li]	3350–3510, 1654,1615,1600, 1542,1505	274,330	245–247
3	2.95(m,2H),3.14(m,2H),3.66 (brd,1H),3.71(m, 1H),3.73 (S,3H),6.56(S,1H),6.71(S,1H), 6.88(d,1H),7.89(d,2H)	56.5,61.6,70.6,73.4,76.1, 76.8,94.2,102.5,104.5, 105.0,115.1,122.0,122.5, 143.0,146.9,147.3,153.3,154.3, 155.8,156.9,157.1,181.2	501[M + Na] 485[M + Li]	3381,1650,1605, 1540,1407,978	255,274,345	265–267
4			414(100),399(45), 395(54),381(42), 329(50),303(70), 275(18)			140–141
5	3.68(S,3H),3.83(S,3H),6.27 (S,1H),6.64(S,1H), 6.94(S,1H),8.02(S,1H)	56.1,56.7,94.0,99.3, 100.8,105.6,110.4,116.4, 121.0,141.5,148.5,153.0,155.5, 158.5,163.3,164.5,181.0	330(100),315(36), 330(48),178(12), 168(8),153(44), 152(14), 135(22)	3464,3246, 1647,1627, 1445	295,266, 259,253	250–252
6			178(100),147(48), 135(72),107(81), 77(85), 51(70)	3247,1656, 1599, 1519	337,305, 238,222	

The IR spectra showed sugar at 3350–3150 cm^{-1} and 1035 cm^{-1}. Its UV, IR, MP and ^{1}H NMR spectral data agreed well with hispidulin-7-*O*-D-glucoside isolated from this herb (Jian et al., 1987). IR (KBr, cm^{-1}): 3350–3150 (H), 1654(C=O), 1615, 1600, 1542, 1505 (C=C). 1050(Glu). The detailed spectral data of hispidulin-7-*O*-D-glucoside were listed in Table 1.

Compound **3** was isolated from $CHCl_3$/acetone (10:1) as yellow needles and was determined as $C_{22}H_{22}O_{11}$ by FAB-MS data (m/z 501 $[M + Na]^+$; 485 $[M + Li]^+$) and by counting carbons and hydrogens from its ^{13}C NMR DEPT. The sugar showed sugar at 3381 cm^{-1} and 1047 cm^{-1}. Its UV, IR, MP and ^{1}H NMR data were identical with those of 6-methoxyluteolin-7-glucoside isolated from *S. plebeia* (Jian et al., 1987). IR (KBr, cm^{-1}), 3381 (OH), 1650(C=O), 1605, 1540(C=C), 1047(Glu), 978. The detailed spectral data of 6-methoxyluteolin-7-glucuonide were listed in Table 1.

Compound **4** was obtained from petroleum/acetone (10:1) as colourless needles, MP: 140–141, $[\alpha]_D$: 37°C (c = 0.02, $CHCl_3$), MS m/z (%): 414(100), 399(45), 396(54), 381(42), 329(50), 303(70), 275(18). These data agreed well with those of β-sitosterol standard. Compound **4** and standard of β-sitosterol had the same R_fs in TLC. The compound was identified as β-sitosterol.

Compound 5 was isolated from benzene/acetone (15:1) as yellow needles and was determined as $C_{17}H_{14}O_7$ by EI-MS data (m/z 330 $[M]^+$) and by counting carbons and hydrogens from its ^{13}C NMR DEPT spectra. The IR and UV spectra indicated the presence of hydroxyl (3464, 3246 cm^{-1}) and aromatic ring (ν_{max}1647, 1627, 1445 cm^{-1} and λ_{max} 266 nm) group. The data were identical with those of 2′-hydroxyl-5′-methoxy biochanin A (Augustin, Dale & Michael, 1989). IR (KBr, cm^{-1}) 3464, 3246(–OH), 1647, 1627, 1445 (C=C). The detailed spectral data of 2′-hydroxyl-5′-methoxy biochanin A are listed in Table 1.

Table 2
Antioxidant effects (Pfs) of compounds isolated from *Salvia plebeia* in lard at l00°C ($n = 4$)

Compound	**1**	**2**	**3**	**4**	**5**	**6**	BHT	α-Tocopherol
0.02%	1.06±0.13	0.95±0.10	4.10±0.35	5.30±0.47	5.2±0.64	0.94±0.08	4.2±0.33	3.72±0.63
0.04%	1.39±0.11	1.07±0.21	7.96±0.68	8.16±0.71	9.03±0.85	1.18±0.13	5.82±0.49	4.21±0.55

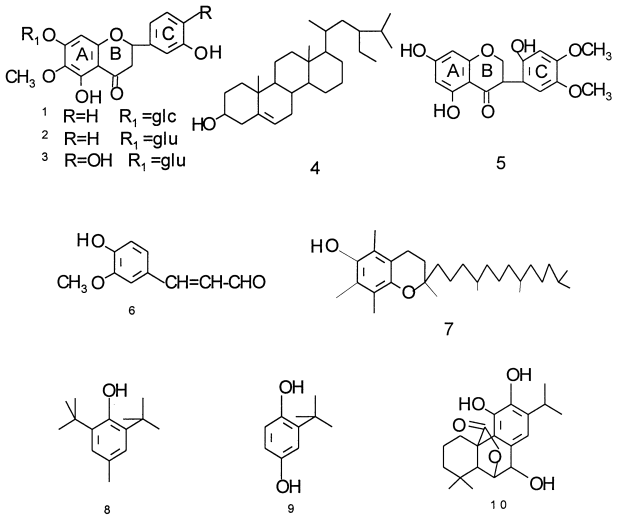
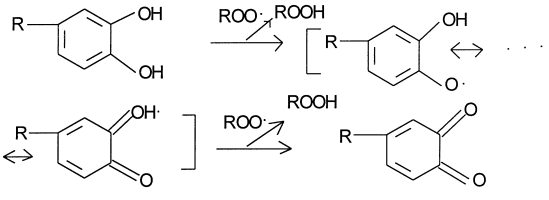


Fig. 1. The structures of compounds separated from *Salvia plebeia* and comparison samples.

Compound **6** was obtained from petroleum/acetone (10:1) as a colourless oil and was determined as $C_{10}H_{10}O_3$ by EI-MS data (*m*/*z* 178[M]$^+$). The data were identical with those of conferyl aldehyde (Kosuge, Mitsunage & Onmoto, 1994). UV^{MeOH} (nm): 222, 238, 305, 337. IR (KBr, cm^{-1}) 3247(−OH), 1656, 1599, 1519(C=C), 1136. EI-MS *m*/*z* (%): 178 (M$^+$, 100), 147 (48), 135(72), 107(81), 77(85), 51(70), 40(48). The detailed spectral data of conferyl aldehyde are listed in Table 1. All six compounds isolated from *Salvia plebeia* were shown in Fig. 1.

Antioxidant activities of compounds **1**–**6** were evaluated using the OSI instrument at 100°C. Their Pfs are listed in Table 2. The results show that compounds **3**, **4** and **5** at 0.02% levels had very strong antioxidant activities, even much stronger than that of α-tocopherol, and compounds **4** and **5** much stronger than those of BHT and compound 3, almost as strong as BHT. At 0.04%, compounds **3**, **4** and **5** were much stronger than those of BHT and α-tocopherol.

The aglycone of compound **3** had only one more hydroxy group than that of compounds **1** and **2** on ring C. Compound **3** had strong antioxidant activity, but compounds **1** and **2** did not show any antioxidant activity. The aglycones of compounds 1 and 2 were hispidulin, and the sugars were different, but their antioxidant activities (Pf) were almost the same. Compound **5** also demonstrated strong antioxidant activity although it did not have any sugar linked with it. This showed that the aglycone was much more important than the sugar in antioxidant activity. In flavones, ring A can effect little antioxidant activity because the ring B condensed with it is strongly a electron-withdrawing. Actually, ring C usually is an antioxidant-active group. If there is an electron donating group, especially a hydroxyl group located on *o*- or *p*- positions of phenolic compounds, their antioxidant activities are increased greatly (Duan, Weng & Dong et al., 1998; Weng, 1993). Also, this is one of the main reasons why *t*-butylatedhydroquinone (TBHQ) and rosmanol (compounds **9** and **10** in Fig. 1) have such strong antioxidant activities because these groups make the phenols more easily donate hydrogen atoms to active free radicals to interrupt the chain reaction of autoxidation. The antioxidant mechanism of *o*-diphenols is shown in Scheme 1. In the same manner as mentioned above, there is a strong withdrawing group, i.e. carbonyl group in conferyl aldehyde, so conferyl aldehyde showed little antioxidant activity.



Scheme 1. Explanation of *O*-diphebolic compounds possessing strong antioxidant effects from their structures.

However, it was interesting that β-sitosterol demonstrated quite strong antioxidant activity. But its antioxidant mechanism is not yet clear.

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