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Phytochemical Constituents from *Salvia plebeia*

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Abstract – Phytochemical investigation of *Salvia plebeia* resulted in the isolation of nine compounds. Their structures were determined to be 6-methoxynaringenin **(1)**, 6-methoxynaringenin-7-*O*-*β*-D-glucoside **(2)**, hispidulin **(3)**, homoplantaginin (**4**), nepetin **(5)**, nepitrin **(6)**, 6-hydroxyluteolin **(7)**, caffeic acid **(8)** and rosmarinic acid **(9)** by spectroscopic analyses. 6-Methoxynaringenin (**1**), 6-hydroxyluteolin **(7)** and rosmarinic acid **(9)** were isolated from this plant for the first time.
Keywords – *Salvia plebeia*, 6-Methoxynaringenin, 6-Hydroxyluteolin, Rosmarinic acid

Introduction

Salvia plebeia R. Br. (Lamiaceae), an annual or biennial plant, distributes in Korea, China and Japan. The whole plant of this species is used in traditional medicines for the treatment of hepatitis, inflammation, and haemorrhoids (Lu and Foo, 2002). Previous phytochemical studies on *S. plebeia* established the occurrence of flavonoids (Weng and Wang, 2000; Xiang *et al*., 2008), diterpenes (Garcia-Alvarez *et al.*, 1986), lignans (Plattner and Powell, 1978; Powell and Plattner, 1976), and caffeic acids derivatives (Jin *et al*., 2008; Jin *et al*., 2009). *S. plebeia* extract was reported to have pharmacological activities including anti-tyrosinase (Kim *et al*., 2003), antioxidant (Lim *et al*., 2007) and anti-anaphylactic effects (Jeong *et al*., 2004). To investigate bioactive compounds from *S. plebeia*, seven flavonoids and two phenolics were isolated from the EtOH (70%) extracts of the whole plants of *S. plebeia*. Structure identification of these compounds was based on spectroscopic analyses and comparing with published data. 6-Methoxynaringenin (**1**), 6-hydroxyluteolin (**7**) and rosmarinic acid (**9**) were isolated from this plant for the first time.

Experimental

General Experimental Procedures – Melting points were determined using an Electrothermal apparatus uncorrected. Ultraviolet absorption spectra were obtained on a CARY 100 UV-Vis spectrophotometer. ESI-MS data were obtained on a Finnigan Navigator mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a VARIAN VNMRS-400 spectrometer operating at 400 MHz for proton and 100 MHz for carbon respectively. The chemical shift values were reported in δ (ppm) relative to the internal standard TMS or residual solvent peak, the coupling constants (*J*) were reported in Hertz (Hz). Silica gel 60 (Merck, 70 - 230 mesh and 230 - 400 mesh) and Sephadex LH-20 (Pharmacia) were used for column chromatography. Silica gel F_{254} plates (Merck) were used for TLC.

Plant material – The herbal samples of *S. plebeia* were bought in September 2009 at kyungdong market, Korea. A voucher specimen (LDSP-2009-1) has been verified by Dr. Kun K. Lee and was deposited at the Coreana Cosmetics Co. Ltd. Songpa R&D Center, Korea.

Extraction and Isolation – The dried and ground *S. plebeia* plants (1.5 kg) were extracted with 70% EtOH at room temperature. The ethanol extract was evaporated under vacuum to yield a dark green residue (140 g), of which 120 g was suspended in H_2O and partitioned with n-hexane, CH_2Cl_2 and EtOAc, successively. The CH_2Cl_2 layer residue (30 g) was chromatographed on a silica gel column by eluting with gradient system of n-hexane : EtOAc (10 : 1 to 1 : 1) to obtain six major fractions (F1-F6). Compound **1** (60 mg) was obtained from fraction F2 by purification on Sephadex LH-20 ($CHCl_3$-MeOH, 1 : 1) and recrystallization from MeOH. Fraction F3 was further separated by repeated Sephadex LH-20 column chromatography (CC) eluting with $CHCl_3$: MeOH (1 : 1) to yield compounds **3** (120 mg), **5** (100 mg) and **8** (20 mg). Fraction

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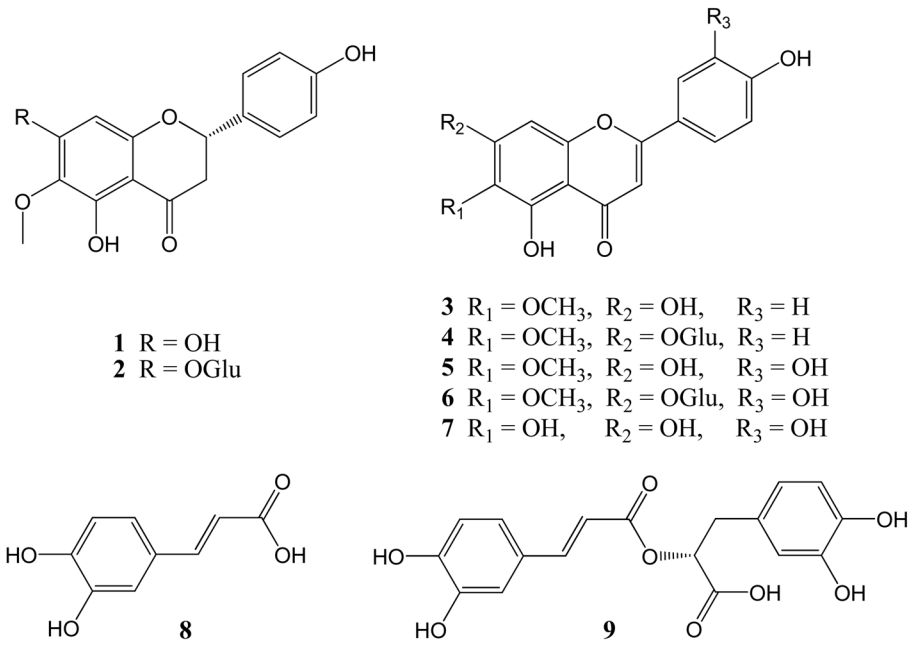

Fig. 1. Structures of compounds **1 - 9** from *S. plebeia*.

F5 afforded **7** (70 mg) by purification on Sephadex LH-20 ($CHCl_3$-MeOH, 1 : 1) and recrystallization from MeOH. The EtOAc layer residue (40 g) was subjected to silica gel column chromatography by eluting with gradient system of EtOAc : MeOH (30 : 1 to 5 : 1) to obtain five major fractions (I-V). Fraction II afforded **9** (50 mg) by CC on silica gel eluting with CH_2Cl_2-MeOH (15 : 1) and Sephadex LH-20 eluting with MeOH. Fraction IV was further separated by repeated Sephadex LH-20 CC eluting with MeOH to yield compounds **2** (40 mg), **4** (100 mg) and **6** (80 mg), which were further purified by recrystallization from MeOH.

6-Methoxynaringenin (1) – Light yellow needles; m.p. 228 - 230 °C; UV (MeOH) λ_{max} nm: 290, 340; ESI-MS *m/z*: 303 $[M+H]^+$; ^{1}H-NMR (CD_3COCD_3, 400 MHz): δ 12.32 (1H, s, 5-OH), 7.38 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.88 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.00 (1H, s, H-8), 5.42 (1H, dd, *J* = 13.2, 2.8 Hz, H-2β), 3.78 (3H, s, OCH_3), 3.18 (1H, dd, *J* = 17.2, 13.2 Hz, H-3α), 2.74 (1H, dd, *J* = 17.2, 2.8 Hz, H-3β); ^{13}C-NMR (CD_3COCD_3, 100 MHz): δ 79.9 (C-2), 43.5 (C-3), 198.0 (C-4), 156.2 (C-5), 130.8 (C-6), 159.7 (C-7), 95.7 (C-8), 159.6 (C-9), 103.3 (C-10), 129.8 (C-1'), 129.0 (C-2'), 116.2 (C-3'), 158.6 (C-4'), 116.2 (C-5'), 129.0 (C-6'), 60.8 (OCH_3).

6-Methoxynaringenin-7-*O*-*β*-D-glucoside (2) – White powder; UV (MeOH) λ_{max} nm: 282, 342; ESI-MS *m/z*: 465 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 11.97 (1H, s, 5-OH), 9.60 (1H, s, 4'-OH), 7.32 (2H, d, *J* = 8.4 Hz, H-2', 6'), 6.79 (2H, d, *J* = 8.4 Hz, H-3', 5'), 6.31 (1H, s, H-8), 5.47 (1H, dd, *J* = 13.2, 2.8 Hz, H-2β), 4.98 (1H, d, *J* = 6.8 Hz, H-1''), 3.68 (3H, s, OCH_3), 3.16 (1H, m, H-3α), 2.72 (1H, dd, *J* = 17.2, 2.8 Hz, H-3β); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 79.4 (C-2), 42.9 (C-3), 198.5 (C-4), 155.1 (C-5), 130.8 (C-6), 159.3 (C-7), 95.2 (C-8), 158.7 (C-9), 103.9 (C-10), 129.4 (C-1'), 129.1 (C-2'), 115.9 (C-3'), 158.5 (C-4'), 115.9 (C-5'), 129.1 (C-6'), 100.5 (C-1''), 73.8 (C-2''), 77.8 (C-3''), 70.2 (C-4''), 77.3 (C-5''), 61.2 (C-6''), 61.0 (OCH_3).

Hispidulin (3) – Yellow powder; UV (MeOH) λ_{max} nm: 274, 334; ESI-MS *m/z*: 301 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 13.08 (1H, s, 5-OH), 10.68 (1H, s, 7-OH), 10.37 (1H, s, 4'-OH), 7.93 (2H, d, *J* = 8.4 Hz, H-2', 6'), 6.93 (2H, d, *J* = 8.4 Hz, H-3', 5'), 6.78 (1H, s, H-3), 6.59 (1H, s, H-8), 3.70 (3H, s, OCH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 164.4 (C-2), 103.0 (C-3), 182.7 (C-4), 153.4 (C-5), 132.0 (C-6), 157.8 (C-7), 94.9 (C-8), 153.0 (C-9), 104.7 (C-10), 121.9 (C-1'), 129.0 (C-2'), 116.6 (C-3'), 161.8 (C-4'), 116.6 (C-5'), 129.0 (C-6'), 60.6 (OCH_3).

Homoplantaginin (4) – Yellow powder; UV (MeOH) λ_{max} nm: 276, 332; ESI-MS *m/z*: 463 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 12.96 (1H, s, 5-OH), 10.41 (1H, s, 4'-OH), 7.95 (2H, d, *J* = 8.8 Hz, H-2', 6'), 7.02 (1H, s, H-8), 6.94 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.88 (1H, s, H-3), 5.11 (1H, d, *J* = 6.4 Hz, H-1''), 3.77 (3H, s, OCH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 165.0 (C-2), 103.4 (C-3),

183.0 (C-4), 152.8 (C-5), 133.2 (C-6), 157.2 (C-7), 95.0 (C-8), 153.2 (C-9), 106.4 (C-10), 121.8 (C-1'), 129.2 (C-2'), 116.7 (C-3'), 162.0 (C-4'), 116.7 (C-5'), 129.2 (C-6'), 100.5 (C-1''), 73.8 (C-2''), 78.0 (C-3''), 70.2 (C-4''), 77.4 (C-5''), 61.3 (C-6''), 61.0 (OCH_3).

Nepetin (5) – Yellow powder; UV (MeOH) λ_{max} nm: 282, 342; ESI-MS *m/z*: 317 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 13.09 (1H, s, 5-OH), 10.70 (1H, s, 7-OH), 7.41 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 7.39 (1H, d, *J* = 2.0 Hz, H-2'), 6.90 (1H, d, *J* = 8.0 Hz, H-5'), 6.67 (1H, s, H-3), 6.59 (1H, s, H-8), 3.75 (3H, s, OCH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 164.6 (C-2), 103.0 (C-3), 182.7 (C-4), 153.4 (C-5), 132.0 (C-6), 157.9 (C-7), 94.7 (C-8), 153.0 (C-9), 104.7 (C-10), 122.2 (C-1'), 114.0 (C-2'), 146.4 (C-3'), 150.3 (C-4'), 116.6 (C-5'), 119.6 (C-6'), 60.6 (OCH_3).

Nepitrin (6) – Yellow powder; UV (MeOH) λ_{max} nm: 284, 340; ESI-MS *m/z*: 479 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 12.98 (1H, s, 5-OH), 7.44 (1H, dd, *J* = 8.4, 2.4 Hz, H-6'), 7.41 (1H, d, *J* = 2.4 Hz, H-2'), 6.90 (1H, d, *J* = 8.4 Hz, H-5'), 6.97 (1H, s, H-8), 6.73 (1H, s, H-3), 5.13 (1H, d, *J* = 7.2 Hz, H-1''), 3.77 (3H, s, OCH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 165.2 (C-2), 103.4 (C-3), 182.9 (C-4), 152.8 (C-5), 133.2 (C-6), 157.2 (C-7), 95.0 (C-8), 153.2 (C-9), 106.4 (C-10), 122.2 (C-1'), 114.2 (C-2'), 146.5 (C-3'), 150.6 (C-4'), 116.6 (C-5'), 119.8 (C-6'), 100.9 (C-1''), 73.9 (C-2''), 77.9 (C-3''), 70.3 (C-4''), 77.4 (C-5''), 61.3 (C-6''), 60.9 (OCH_3).

6-Hydroxyluteolin (7) – Yellow powder; UV (MeOH) λ_{max} nm: 276, 338; ESI-MS *m/z*: 303 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 12.80 (1H, s, 5-OH), 10.45 (1H, s, 7-OH), 7.41 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 7.39 (1H, d, *J* = 2.0 Hz, H-2'), 6.88 (1H, d, *J* = 8.0 Hz, H-5'), 6.63 (1H, s, H-3), 6.54 (1H, s, H-8); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 164.3 (C-2), 102.9 (C-3), 182.6 (C-4), 147.3 (C-5), 129.8 (C-6), 153.9 (C-7), 94.4 (C-8), 150.1 (C-9), 104.6 (C-10), 122.5 (C-1'), 113.9 (C-2'), 146.4 (C-3'), 150.3 (C-4'), 116.6 (C-5'), 119.5 (C-6').

Caffeic acid (8) – Yellow powder; UV (MeOH) λ_{max} nm: 235, 290, 320; ESI-MS *m/z*: 181 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.53 (1H, d, *J* = 16.0 Hz, H-7), 7.03 (1H, d, *J* = 2.0 Hz, H-2), 6.93 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.77 (1H, d, *J* = 8.0 Hz, H-5), 6.22 (1H, d, *J* = 16.0 Hz, H-8); ^{13}C-NMR (CD_3OD, 100 MHz): δ 126.6 (C-1), 115.4 (C-2), 146.0 (C-3), 148.2 (C-4), 121.8 (C-5), 114.3 (C-6), 145.6 (C-7), 114.0 (C-8), 170.0 (C-9).

Rosmarinic acid (9) – White powder; UV (MeOH) λ_{max} nm: 230, 329; ESI-MS *m/z*: 361 $[M+H]^+$; ^{1}H-NMR (CD_3COCD_3, 400 MHz): δ 7.57 (1H, d, *J* = 16.0 Hz, H-7'), 7.17 (1H, d, *J* = 2.0 Hz, H-2'), 7.05 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 6.86 (1H, d, *J* = 8.0 Hz, H-5'), 6.85 (1H, d, *J* = 2.0 Hz, H-2), 6.75 (1H, d, *J* = 8.0 Hz, H-5), 6.68 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.30 (1H, d, *J* = 16.0 Hz, H-8'), 5.23 (1H, dd, *J* = 8.4, 4.4 Hz, H-8β), 3.13 (1H, dd, *J* = 14.0, 4.4 Hz, H-7β), 3.04 (1H, dd, *J* = 14.0, 8.4 Hz, H-7α); ^{13}C-NMR (CD_3COCD_3, 100 MHz): δ 127.4 (C-1), 115.3 (C-2), 145.7 (C-3), 146.2 (C-4), 121.7 (C-5), 116.4 (C-6), 37.4 (C-7), 73.7 (C-8), 171.3 (C-9), 129.1 (C-1'), 114.9 (C-2'), 144.8 (C-3'), 146.6 (C-4'), 122.8 (C-5'), 116.0 (C-6'), 148.9 (C-7'), 117.4 (C-8'), 166.9 (C-9').

Results and Discussion

Column chromatographic separation of the CH_2Cl_2- and EtOAc- soluble fractions of the ethanol extract of *S. plebeia* with silica gel and Sephadex LH-20 led to the isolation of seven flavonoids (**1 - 7**) and two phenolics (**8 - 9**).

The structures of **2 - 6** and **8** were identified to be 6-methoxynaringenin-7-*O*-*β*-D-glucoside (**2**), hispidulin (**3**), homoplantaginin (**4**), nepetin (**5**), nepitrin (**6**) (Nagao *et al*., 2002; Xiang *et al*., 2008), and caffeic acid (**8**) (Jeong *et al*., 2008) by comparing of ^{1}H-, ^{13}C-NMR and MS data with the literatures.

Compound **1** was obtained as light yellow needles, the ^{1}H- and ^{13}C-NMR spectra showed that it is a flavanone with three hydroxy groups and one methoxy group. Its ^{1}H-NMR spectrum showed the typical ABX system of three protons (δ 2.74 (1H, dd, *J* = 17.2, 2.8 Hz), 3.18 (1H, dd, *J* = 17.2, 13.2 Hz), 5.42 (1H, dd, *J* = 13.2, 2.8 Hz)) corresponding to the C-ring in a flavanone. The ^{1}H-NMR resonances of two doublets at 7.38 and 6.88 ppm (each, 2H, d, *J* = 8.8 Hz) indicated *para*-substitution in the B-ring. The peak at *m/z* 120 in the MS, arising from RDA fragmentation of the C-ring, confirmed the presence of a B-ring hydroxyl. The remaining two hydroxyls and one methoxyl are thus located on the A-ring, and the observed singlet at 6.00 ppm indicated H-8. Thus, **1** was identified as 5,7,4'-trihydroxy-6-methoxyflavanone (6-methoxynaringenin) by comparing the spectral data with those reported in the literature (Wollenweber *et al*., 1993; Xiang *et al*., 2008). 6-Methoxynaringenin (**1**) was isolated from this plant for the first time.

Compound **7** was obtained as yellow powder, the ^{1}H-NMR spectrum suggested **7** to be a 5,6,7,3',4'-penta-*O*-substituted flavone derivative, showing the signals due to two aromatic singlets at (δ 6.63 (1H, s, H-3), 6.54 (1H, s, H-8)) and B-ring protons at (δ 7.41 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 7.39 (1H, d, *J* = 2.0 Hz, H-2'), 6.88 (1H, d, *J* = 8.0 Hz, H-5')). **7** showed the ^{1}H- and ^{13}C-NMR data

similar to those of **5**, except of one more methyl group in **5**. Thus, compound **7** was identified as 5,6,7,3',4'-pentahydroxyflavone (6-hydroxyluteolin), which was isolated from this plant for the first time.

Compound **9** was obtained as white powder, the ^{1}H-NMR spectra indicated the presence of one *trans* olefinic protons (δ 7.57 (1H, d, J = 16.0 Hz, H-7'), 6.30 (1H, d, J = 16.0 Hz, H-8')), two 1,3,4-trisubstituted phenyl groups (δ 7.17 (1H, d, J = 2.0 Hz, H-2'), 7.05 (1H, dd, J = 8.0, 2.0 Hz, H-6'), 6.86 (1H, d, J = 8.0 Hz, H-5'); 6.85 (1H, d, J = 2.0 Hz, H-2), 6.75 (1H, d, J = 8.0 Hz, H-5), 6.68 (1H, dd, J = 8.0, 2.0 Hz, H-6)), and the typical ABX system of three protons (δ 3.04 (1H, dd, J = 14.0, 8.4 Hz, H-7α), 3.13 (1H, dd, J = 14.0, 4.4 Hz, H-7β), 5.23 (1H, dd, J = 8.4, 4.4 Hz, H-8β)). The ^{1}H- and ^{13}C-NMR spectra revealed the signals due to a caffeoyl group and a 3,4-dihydroxyphenyl lactic acid moiety. Thus, **9** was identified as rosmarinic acid by comparing the spectral data with those reported in the literature (Satake *et al*., 1999). Rosmarinic acid (**9**) was isolated from this plant for the first time.

References

Garcia-Alvarez, M.C., Hasan, M., Michavila, A., Fernandez-Gadea, F., and Rodriguez, B., Epoxysalviacoccin, a neo-clerodane diterpenoid from *Salvia plebeia*. *Phytochemistry* **25**, 272-274 (1986).

Jeong, D.M., Jung, H.A., Kang, H.S., and Choi, J.S., Peroxynitrite scavengers from *Phellinus linteus*. *Nat. Prod. Sci*. **14**, 1-11 (2008).

Jeong, H.J., Hong, S.H., and Kim, H.M., Oriental medicines with anti-anaphylactic effect. *Oriental Pharmacy and Experimental Medicine* **4**, 125-136 (2004).

Jin, Q., Han, X.H., Hwang, J.H., Hong, S.S., Park, M., Lee, C., Lee, C.H., Lee, D., Lee, M.K., and Hwang, B.Y., A new phenylbutanone glucoside from *Salvia plebeian*. *Nat. Prod. Sci.,* **15**, 106-109 (2009).

Jin, X.F., Lu, Y.H., Wei, D.Z., and Wang, Z.T., Chemical fingerprint and quantitative analysis of *Salvia plebeia* R.Br. by high-performance liquid chromatography. *J. Pharm. Biomed. Anal*. **48**, 100-104 (2008).

Kim, S.J., Heo, M.Y., Son, K.H., and Kim, H.P., Tyrosinase inhibitory activity of 80 plant extracts (II). *The J. of Applied Pharmacology* **11**, 5-7 (2003).

Lim, J.A., Yun, B.W., and Baek, S.H., Antioxidative activity and nitrite scavenging ability of methanol extract from *Salvia plebeia* R. Br. *Kor. J. Medicinal Crop Sci*. **15**, 183-188 (2007).

Lu, Y., and Foo, L.Y., Polyphenolics of *Salvia* - a review. *Phytochemistry* **59**, 117-140 (2002).

Nagao, T., Abe, F., Kinjo, J., and Okabe, H., Antiproliferative constituents in plants 10 flavones from the leaves of *Lantana montevidensis* Briq. and consideration of structure-activity relationship. *Biol. Pharm. Bull.* **25**, 875-879 (2002).

Plattner, R.D. and Powell, R.G., A secoisolariciresinol branched fatty diester from *Salvia plebeia* seed. *Phytochemistry* **17**, 149-150 (1978).

Powell, R.G., and Plattner, R.D., Structure of a secoisolariciresinol diester from *Salvia plebeia* seed. *Phytochemistry* **15**, 1963-1965 (1976).

Satake, T., Kamiya, K., Saiki, Y., Hama, T., Fujimoto, Y., Kitanaka, S., Kimura, Y., Uzawa, J., Endang, H., and Umar, M., Studies on the constituents of fruits of *Helicteres isora* L. *Chem. Pharm. Bull.* **47**, 1444-1447 (1999).

Weng, X.C. and Wang, W., Antioxidant activity of compounds isolated from *Salvia plebeia*. *Food. Chem*. **71**, 489-493 (2000).

Wollenweber, E., Mann, K., Arriaga-Giner, F.J., Roitman, J.N., and West, J.G., Exudate flavonoids from two Australian Asteraceae, *Bracteantha viscosa* and *Cassinia quinquefaria*. *Phytochemistry* **33**, 871-873 (1993).

Xiang, L., Chen, H.N., Xu, C.M., Zhang, S.S., and Wang, H.Y., Study on flavanoids from *Salvia plebeian*. *Chin. Pharm. J*. **43**, 813-815 (2008).

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