

## Flavonoids from *Feronia Limonia*

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**Abstract:** Six known flavones and one flavanone glycoside were isolated from the aerial parts of *Feronia limonia* (Rutaceae). The structures of the flavanone glycoside was characterized as 5-hydroxy-2-(4'-hydroxyphenyl)-2'',2''-dimethyl-6-[3-methylbut-2-ene]-2, 3-dihydro-pyrano-chromen-4-one -4'-O- $\beta$ -D-glucopyranoside. The structures of the compounds were elucidated by spectral analysis.

**Keywords:** Rutaceae, *Feronia lemonia*, Flavanoids

### 1. Introduction

*Feronia limonia* Swingle [*Feronia elephantum* Correa / *Limonia acidissima* L. / *Schinus limonia* L.] belongs to the family Rutaceae. Vernacular names include wood apple, elephant apple, monkey fruit, curd fruit, and *kath bel* or a variety of other dialectal names in its native area from India [1]. It is one of the important medicinal plants commonly found in Shahjahanpur district. This plant is prescribed as a traditional medicine for the treatment of various ailments [1-10].

### 2. Experimental

#### 2.1. General Procedures

Ultra violet absorption spectra were recorded on a Perkin-Elmer Lambda Bio 20 UV spectrometer. The IR spectra were obtained on a Perkin-Elmer 1710 infrared Fourier transform spectrometer using the KBr disc method. NMR spectra were recorded on Bruker AVANCE DRX-300 (300, 75 MHz). Chemical shifts are shown in  $\delta$  values (ppm) with tetramethylsilane (TMS) as an internal reference. FAB-MS spectra were recorded on JEOL SX 1021/DA-6000 mass spectrometer. Column chromatography was carried out using silica gel (60-120 mesh). Chemicals are of analytical-reagent grade and were purchased from E-Merck (India).

#### 2.2. Plant Material

The aerial parts of *Feronia limonia* were collected from the rural areas of the Shahjahanpur district in the month of November. Authentication was achieved by the comparison with the herbarium specimen (GFBD 1698) deposited in the herbarium of the faculty of botany, G. F. College (Rohilkhand University), Shahjahanpur. Freshly harvested and dried material was used since old dried material stored for a period may undergo some qualitative changes. Healthy aerial parts were spread out and dried in the

laboratory at room temperature until they broke easily by hand.

#### 2.3. Extraction and Isolation

Air-dried and powdered aerial parts of *Feronia limonia* (1.2 kg) were grounded using a grinder, and extracted successively with petrol-ether, dichloromethane and ethyl acetate (3L x 5 times each) using a Soxhlet apparatus. Out of these extracts only  $\text{CH}_2\text{Cl}_2$  and ethyl acetate extracts were considered for further examination. Evaporation of the  $\text{CH}_2\text{Cl}_2$  extract in vacuo provided 6.32 g of residue. A well-stirred suspension of silica gel (100 -150 g in petroleum ether 60-80<sup>0</sup>) was poured into column (150 cm long and 50 mm in diameter). When the absorbent was settled, the excess of petrol-ether was allowed to pass through column. A portion of this oily material (2.5 g) was subjected to column chromatographic separation and eluted with a mobile phase of increasing polarity: petroleum-ether, chloroform, acetone, ethyl acetate and methanol. Elution with petroleum ether:  $\text{CHCl}_3$  (3:7) afforded a yellow powder (**FL-2**, 1.23 mg), similarly on eluting with petroleum ether:  $\text{CHCl}_3$  (5:2),  $\text{CHCl}_3$ : EtOAc (2:8), acetone: EtOAc (5:3), acetone: EtOAc (4:5), EtOAc: MeOH (6:8) yielded compounds **FL-3** (1.14 mg), (**FL-4**, 0.69 mg), (**FL-5**, 0.58 mg), (**FL-6**, 1.01 mg), and (**FL-7**, 0.23 mg) respectively. Correspondingly the EtOAc extract (11.2 g) chromatographed over a silica gel column eluted with  $\text{CHCl}_3$ : MeOH (8: 2) furnished **FL-1** (1.17 mg).

**Compound FL-1:** yellow amorphous powder (1.17 mg); UV (MeOH)  $\lambda_{\text{max}}$ : 218, 272 and 363 nm; MS ( $m/z$ , %): 568  $[\text{M}]^+$  (100), 554  $[\text{M}-\text{CH}_3]^+$  (57), 525  $[\text{M}-\text{CH}_3-\text{CO}]^+$  (94), 405  $[\text{M}-\text{Glc.}]^+$  (57), 350  $[\text{M}-\text{C}_4\text{H}_7]^+$  (41), 336 (14), 203(17) and 55(11); IR (KBr)  $\nu_{\text{max}} \text{ cm}^{-1}$ : 3410, 2920, 1626, 1519, 1592, 1450, 1378, 1190, 1165, 1110, 836 and 573; <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$ : 12.34 (br. s., 1H, 5-OH), 7.29 (d, 2H,  $J=8.2$  Hz, H-2'/ 6'), 6.81 (d, 2H,  $J=8.2$  Hz, H-3'/ 5'), 6.48 (d, 1H,  $J=10.0$  Hz, H-4''), 5.39 (d, 1H,  $J=10.0$  Hz, H-3''), 5.29 (dd, 1H,  $J=13.0$ , 3.0 Hz, H-2), 5.22 (m, 1H, H-2''), 3.59 (1H,  $m$ , Glc., H-5), 3.68-3.89 (2H,  $m$ , Glc., H-6), 5.10(1H,  $br.s.$ , anomeric proton, Glc., H-1), 3.33 - 3.54(3H,

m, Glc.), 3.29 (d, 2H,  $J=7.3$  Hz, H-1'''), 3.00 (dd, 1H,  $J=17.0, 13.0$  Hz, H-3a), 2.71 (dd, 1H,  $J=17.0, 3.0$  Hz, H-3b), 1.82 (s, 3H, 5'''-CH<sub>3</sub>), 1.66 (s, 3H, 4'''-CH<sub>3</sub>), 1.41 (s, 3H, 5''-CH<sub>3</sub>), 1.39 (s, 3H, 6''-CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$ : 190.0 (C-4), 159.9 (C-7), 156.6 (C-4'), 154.9 (C-9), 153.9 (C-5), 131.7 (C-3''), 130.5 (C-1'), 127.9 (C-2'), 127.9 (C-6'), 125.8 (C-3'''), 122.8 (C-2''), 115.9 (C-4''), 115.3 (C-3'), 115.3 (C-5'), 109.5 (C-6), 102.6 (C-1'''), 102.3 (C-10), 100.9 (C-8), 78.3 (C-2), 77.8 (C-2'''), 76.4 (C-5'''), 76.2 (C-3'''), 72.9 (C-2'''), 69.5 (C-4'''), 61.2 (C-6'''), 43.0 (C-3), 28.8 (C-5''), 28.3 (C-6''), 25.6 (C-4'''), 20.8 (C-1'''), 17.6 (C-5''')

**Compound FL-2:** 5,6,7,8,4'-pentamethoxyflavone (1.23 mg); MS:  $m/z$  372 [M]<sup>+</sup> (34), 357 [M-CH<sub>3</sub>]<sup>+</sup> (56), 341 [M-OCH<sub>3</sub>]<sup>+</sup> (100); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 7.84 (2H, d,  $J=9.2$  Hz, H-2'/H-6'), 7.00 (2H, d,  $J=9.2$  Hz, H-3'/H-5'), 6.63 (1H, s, H-3), 4.11 (3H, s, 5-OMe), 4.01 (3H, s, 8-OMe), 3.91 (6H, s, 6 and 7-OMe), 3.87 (3H, s, 4'-OMe); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$ : 177.3 (C-4), 162.2 (C-2), 161.1 (C-4'), 151.3 (C-7), 148.4 (C-8), 147.7 (C-9), 144.0 (C-5), 138.1 (C-6), 127.8 (C-2'/C-6'), 123.8 (C-1'), 114.8 (C-10), 114.4 (C-3' and C-5'), 106.7 (C-3), 62.3 (5-OMe), 62.1 (6-OMe), 61.9 (7-OMe), 61.7 (8-OMe), 55.5 (4'-OMe).

**Compound FL-3:** 5,6,7,8,3',4'-hexamethoxyflavone (1.14 mg); MS:  $m/z$  402 [M]<sup>+</sup> (26), 387 [M-CH<sub>3</sub>]<sup>+</sup> (58), 371 [M-OCH<sub>3</sub>]<sup>+</sup> (100); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 7.57 (1H, dd,  $J=2.0, 9.0$  Hz, H-6'), 7.41 (1H, d,  $J=2.0$  Hz, H-2'), 6.99 (1H, d,  $J=9.0$  Hz, H-5'), 6.62 (1H, s, H-3), 4.11 (3H, s, 5-OMe), 4.03 (3H, s, 8-OMe), 3.98 (3H, s, 6-OMe), 3.97 (3H, s, 7-OMe), 3.96 (6H, s, 3', 4'-OMe); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$ : 177.4 (C-4), 161.0 (C-2), 151.9 (C-4'), 151.4 (C-7), 149.3 (C-3'), 147.7 (C-9), 144.0 (C-5), 138.0 (C-6 and C-8), 124.0 (C-1'), 119.6 (C-6'), 114.8 (C-10), 111.0 (C-5'), 108.7 (C-2'), 106.7 (C-3), 62.3 (5-OMe), 62.0 (6-OMe), 61.8 (7-OMe), 61.7 (8-OMe), 56.1 (3'-OMe), 56.0 (4'-OMe).

**Compound FL-4:** 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone (0.69 mg);  $m/z$  388 [M]<sup>+</sup> (100), 373 [M-CH<sub>3</sub>]<sup>+</sup> (96), 357 [M-OCH<sub>3</sub>]<sup>+</sup> (55), 343 [M-CH<sub>3</sub>-OCH<sub>3</sub>]<sup>+</sup> (6.4). UV (CH<sub>3</sub>OH)  $\lambda_{max}$  nm: 205, 251, 280, 337; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 7.58 (1H, dd,  $J=2.3$  and  $9.2$  Hz, H-6'), 7.42 (1H, d,  $J=2.3$  Hz, H-2'), 6.99 (1H, d,  $J=9.2$  Hz, H-5'), 6.61 (1H, s, H-3), 4.12 (3H, s, 8-OMe), 3.99 (6H, s, 3', 4'-OMe), 3.97 (3H, s, 7-OMe), 3.96 (3H, s, 6-OMe); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$ : 179.3 (C-4), 158.8, (C-2), 152.9 (C-7), 149.1 (C-5), 149.0 (C-4'), 145.6 (C-3'), 142.9 (C-9), 136.2 (C-6), 132.9 (C-8), 123.7 (C-1'), 121.6 (C-6'), 114.6 (C-2'), 110.5 (C-5'), 107.5 (C-10), 106.5 (C-3), 62.1 (6-OMe), 61.7 (7-OMe), 61.2 (8-OMe), 56.1 (3'-OMe), 56.0 (4'-OMe).

**Compound FL-5:** 4'-hydroxy-5,6,7,8,3'-pentamethoxyflavone (0.58 mg);  $m/z$  388 [M]<sup>+</sup> (14), 373 [M-CH<sub>3</sub>]<sup>+</sup> (100), 357 [M-OCH<sub>3</sub>]<sup>+</sup> (10); UV (CH<sub>3</sub>OH)  $\lambda_{max}$  nm: 248, 268, 272, 340; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 7.52 (1H, dd,  $J=1.5$  and  $9.0$  Hz, H-6'), 7.39 (1H, d,  $J=1.5$  Hz, H-2'), 7.04 (1H, d,  $J=9.0$  Hz, H-5'), 6.60 (1H, s, H-3), 4.04 (3H, s, 8-OMe), 4.02 (3H, s, 5-OMe), 3.99 (3H, s, 3'-OMe), 3.96 (6H, s, 6 and 7-OMe); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$ : 177.4 (C-4), 161.0, (C-2), 151.9 (C-4'), 151.4 (C-7), 149.3 (C-3'), 147.7 (C-9), 144.0 (C-5), 106.7 (C-3), 138.0 (C-6, C-8), 124.0 (C-1'), 119.6 (C-6'), 114.8 (C-10), 111.0 (C-5'), 108.7 (C-

2'), 62.3 (5-OMe), 62.0 (6-OMe), 61.8(7-OMe), 61.7 (8-OMe), 56.1, (3'-OMe).

**Compound FL-6:** 4', 5, 6-Trihydroxy-3',7-dimethoxyflavone (1.01 mg); Yellow crystals; mp 253–255 °C; MS  $m/z$ : 330 [M]<sup>+</sup> (100), 312 (29), 284 (34), 248 (7), 148 (7), 69 (8); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 12.63 (1H, brs, 5-OH), 7.60 (1H, dd,  $J=7.4$  Hz, 2.2 Hz, H-6'), 7.58 (1H, d,  $J=2.2$  Hz, H-2'), 6.95 (1H, d,  $J=7.4$  Hz, H-5'), 6.93 (1H, s, H-8), 6.91 (1H, s, H-3), 3.92 (3H, s, OMe), 3.90 (3H, s, OMe); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$ : 183.1(C-4), 164.6 (C-2), 155.2(C-7), 150.5(C-9), 147.1(C-5), 130.8(C-6), 105.9(C-10), 103.8(C-3), 92.0(C-8).

**Compound FL-7:** 5-hydroxy-7, 3', 4', 5'-tetramethoxyflavanone (0.23 mg); m.p. 160-162 °C; EI-MS ( $m/z$ , %): 361 [M]<sup>+</sup>(22), 360 (69), 330 (100), 179 (13), 167 (5), 164 (22), 151 (43), 95 (11); UV (MeOH)  $\lambda_{max}$  nm: 285, 320; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 2.80 (1H, dd,  $J=3.2, 17.1$  Hz, H-3eq), 3.5 (1H, dd,  $J=13.2, 17.1$  Hz, H-3ax), 3.82 (3H, s, OCH<sub>3</sub>), 3.87 (3H, s, OCH<sub>3</sub>), 3.90 (6H, s, 2x -OCH<sub>3</sub>), 5.35 (1H, dd,  $J=3.2, J=13.2$  Hz, H-2), 6.04 (2H, s, H-6'/H-8), 6.73 (2H, s, H-2'/H-6') and 12.02 s (1H, s, 5-OH)

### 3. Results and Discussion

Compound **FL-1** was obtained as yellow powder from ethyl acetate extract by eluting the column with benzene: chloroform (2: 8). The compound gave a purple colored spot on TLC, when examined under UV light. The compound showed positive test for sugar and flavonoid moiety suggested that the compound may be a flavanoid glycoside [11, 12]. The compound gave a molecular ion peak in its mass spectrum at  $m/z$  568 corresponding to the molecular formula C<sub>31</sub>H<sub>36</sub>O<sub>10</sub>. The IR spectrum of the compound exhibited absorption band for hydroxyl group at 3410 cm<sup>-1</sup>, conjugated carbonyl group at 1625 and 1592 cm<sup>-1</sup> aromatic ring vibrations at 1519, 1440 and 1378 cm<sup>-1</sup> and of ether function at 1194 and 1165 cm<sup>-1</sup>. The U.V. spectrum of the compound illustrated bands at 218, 272, 363 nm, characteristic of a flavanone [13]. Thus, UV and IR absorption bands of compound clearly designate that the compound belongs to the class of flavanone.

The <sup>1</sup>H NMR spectrum of the compound clearly demonstrated the signals of aromatic, 4-pyrone ring, 2H-pyran ring and prenyl group protons. Three one proton double doublets observed at  $\delta$  2.71( $J=17.0, 3.0$  Hz), 3.0( $J=17.0, 13.0$  Hz) and 5.29( $J=13.0, 3.0$  Hz) were assigned to two methylene protons at H-3 and one methine proton H-2 of pyron ring respectively. Two doublets of two protons each of AA', BB' pattern appeared at  $\delta$  7.29( $J=8.2$  Hz) and 6.81 ( $J=8.2$  Hz) were ascribed to ring B protons H-2', H-6' and H-3', H-5' respectively [13]. Two one proton doublets of AB pattern observed at  $\delta$  5.39 ( $J=10$  Hz) and 6.48( $J=10$  Hz) were assigned to H-3'' and H-4'' protons of pyran ring. Two sharp singlets of three protons each at  $\delta$  1.41 and 1.39 were ascribed for two methyl groups at C-2''. Another three protons singlet at  $\delta$  1.66 and 1.82 were assigned to the methyl groups of prenyl side chain. A two protons doublets at  $\delta$  3.29 ( $J=7.3$  Hz) and a one proton

triplet at  $\delta$  5.22 were assigned to H-1''' and H-2''' proton of side chain respectively.

Acid hydrolysis of compound with 2N HCl followed by the usual workup yielded 5-hydroxy-2-(4'-hydroxyphenyl)-2'', 2''-dimethyl-6-[3-methyl-but-2-ene]-2, 3-dihydro-pyrano-chromen-4-one as the only aglycone and glucose. The sugar was identified as D-glucose when compared with authentic sample by Co-PC. The  $^1\text{H}$  NMR spectrum of this compound displayed a signal for an anomeric proton at  $\delta$  5.1 (1H, *d*,  $J = 5.6$  Hz), showing the  $\beta$ - configuration of the sugar [14, 15]. Other sugar protons appeared in the range of  $\delta$  3.33 - 3.54. The anomeric carbon signaled at  $\delta$  102.6 in its  $^{13}\text{C}$  NMR spectrum indicating the 4'-O- $\beta$ -D-glucopyranoside structure of the compound [16]. In the heteronuclear multiple bond connectivity (HMBC) spectrum, a long-range correlation peak was observed from the anomeric proton of substituted glucosyl moiety at  $\delta_{\text{H}}$  5.10 to the 4'-carbon of the aglycon part at  $\delta_{\text{C}}$  156.6. Thus, the position of the glucosyl moiety was confirmed to be at the 4'-position. Thus on the basis of the above spectral evidences the structure of the isolated compound **FL-1** was finally concluded to be 5-hydroxy-2-(4'-hydroxyphenyl)-2'',2''-dimethyl-6-[3-methylbut-2-ene]-2,3-dihydro-pyrano-chromen-4-one-4'-O- $\beta$ -D-glucopyranoside.

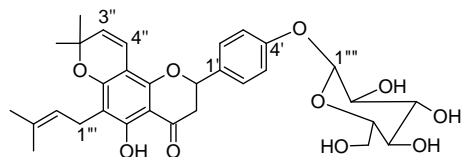


Figure 1. FL-1

Compound **FL-2** was determined to be 5, 6, 7, 8, 4'-pentamethoxyflavone. It had a molecular ion peak  $[M]^+$  at  $m/z$  372 in the mass spectrum that, along with the  $^{13}\text{C}$  NMR spectrum, suggested a molecular formula  $\text{C}_{20}\text{H}_{20}\text{O}_7$ .  $^{13}\text{C}$  NMR spectrum showed signals for eighteen carbons five methoxyls, three methines, and seven quaternary carbons.  $^1\text{H}$  NMR spectrum showed a pair of doublets at  $\delta$  7.84 and 7.00 attributed to H-2'/H-6' and H-3'/H-5', a singlet at  $\delta$  6.63 attributed to H-3, three singlets at  $\delta$  4.11, 4.01, and 3.87 each integrated for three protons, and a singlet at  $\delta$  3.91 integrated for six protons. These data were in agreement with those previously reported for 5, 6, 7, 8, 4'-pentamethoxyflavone [11-16].

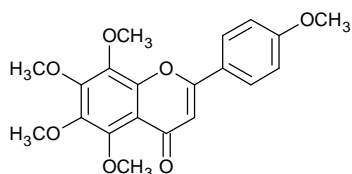


Figure 2. FL-2

Compound **FL-3** was identified as 5, 6, 7, 8, 3', 4'-hexamethoxyflavone. In the EI mass spectrum a molecular ion peak  $[M]^+$  at  $m/z$  402 defined the molecular formula  $\text{C}_{21}\text{H}_{22}\text{O}_8$ .  $^1\text{H}$  NMR spectrum of this compound evidenced

the presence of a 1, 2, 4-trisubstituted aromatic ring, a singlet at  $\delta$  6.62, and six methoxyl groups. The spectral data were close with those previously reported for 5, 6, 7, 8, 3', 4'-hexamethoxyflavone [11-16].

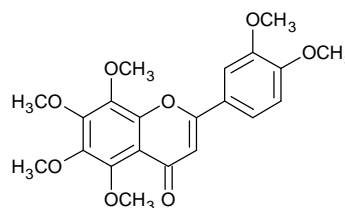


Figure 3. FL-3

Compound **FL-4** was identified as 5-hydroxy-6, 7, 8, 3', 4'-pentamethoxyflavone. The NMR data of compound **FL-4** were similar to that of compound **FL-3**. The  $^1\text{H}$  NMR spectra showed two doublets and a double doublet of a 1, 2, 4-trisubstituted aromatic ring, one singlet at  $\delta$  6.60, and five methoxyls.  $^{13}\text{C}$  NMR spectrum showed twenty carbons defined as five methoxyls, four methines and eleven quaternary carbons. All spectroscopic data agreed with those reported for 5-hydroxy-6, 7, 8, 3', 4'-pentamethoxyflavone [11-16].

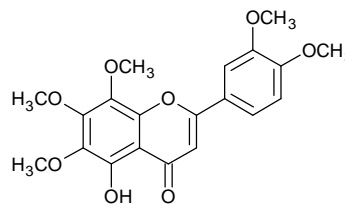


Figure 4. FL-4

Compound **FL-5** was identified as 4'-hydroxy-5, 6, 7, 8, 3'-pentamethoxyflavone on the basis of its spectroscopic data and by comparing them to data reported in the literature [11-16]. The  $^1\text{H}$  NMR spectrum showed two doublets and a double doublet in the aromatic region, one singlet at  $\delta$  6.60, and five methoxyls. The  $^{13}\text{C}$  NMR spectrum showed the presence of five methoxyls, four methines and eleven quaternary carbons [11-16].

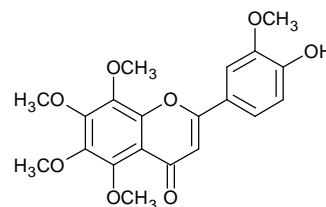


Figure 5. FL-5

Compound **FL-6** was identified as 4', 5, 6-Trihydroxy-3', 7-dimethoxyflavone on the basis of its spectroscopic data and by comparing them to data reported in the literature [17-18]. The  $^1\text{H}$  NMR spectrum showed two doublets and a double doublet in the aromatic region, one singlet at  $\delta$  6.93, and two methoxyls. The  $^{13}\text{C}$  NMR

spectrum showed the presence of two methoxyls, four methines and eleven quaternary carbons [11-16].

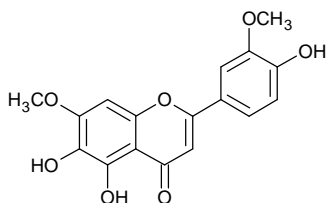


Figure 6. FL-6

Compound **FL-7** was characterized as 5-hydroxy-7, 3', 4', 5'-tetramethoxyflavanone. The MS spectrum for compound **FL-7** shows the molecular formula  $C_{19}H_{20}O_7$ . The  $^1H$ -NMR spectrum shows the presence of a flavanone with four methoxy groups and a 5-OH. In addition two singlets at 6.04 (2H), and 6.73 (2H) were assigned to H-6, H-8 and H-2', H-6' respectively. The spectroscopic data were very close to those reported in the literature for 5-hydroxy-7,3',4',5'-tetramethoxyflavanone [11-16]

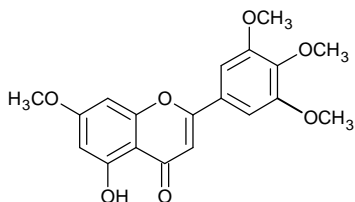


Figure 7. FL-7

#### 4. Conclusions

At our best knowledge, this is the first report describing the isolation of 5-hydroxy-2-(4'-hydroxyphenyl)-2'',2''-dimethyl-6-[3-methylbut-2-ene]-2,3-dihydro-pyrano-chromen-4-one-4'-O- $\beta$ -D-glucopyranoside from this plant.

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