

Postprint: Chemical Constituents of *Sabia parviflora* Leaves

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Abstract

Currently, studies on the chemical constituents and pharmacological activities of *Sabia parviflora* are scarce. To elucidate the material basis of this plant, the present study conducted separation and purification on the dried leaves of *Sabia parviflora* by means of repeated silica gel column chromatography, Sephadex LH-20 column chromatography, preparative thin-layer chromatography, and recrystallization. The structures of the compounds were identified using chemical analysis and spectroscopic methods. The results demonstrated that twelve compounds were isolated from the methanol ultrasonic extract of the dried leaves of *Sabia parviflora*, namely N-trans-feruloyltyramine (1), N-cis-feruloyltyramine (2), N-trans-p-coumaroyltyramine (3), N-cis-p-coumaroyltyramine (4), N-trans-p-coumaroyloctopamine (5), N-cis-p-coumaroyloctopamine (6), ferulic acid (7), apigenin (8), luteolin (9), caffeic acid (10), 5-oxoaporphine (11), and oleanolic acid (12). Among them, compounds 2 and 4-9 were obtained from the genus *Sabia* for the first time, while compounds 1, 3, and 10 were isolated from this species for the first time.

Full Text

Chemical Constituents from the Leaves of *Sabia parviflora*

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Abstract

Current research on the chemical constituents and pharmacological activities of *Sabia parviflora* remains limited. To elucidate the material basis of this medicinal plant, the dried leaves of *Sabia parviflora* were subjected to systematic separation and purification using repeated silica gel column chromatography, Sephadex LH-20 column chromatography, preparative thin-layer chromatography, and recrystallization. The structures of isolated compounds were identified through chemical analysis and spectroscopic methods. Twelve compounds were obtained from the methanol ultrasonic extract of *S. parviflora* leaves: *N*-trans-feruloyltyramine (1), *N*-cis-feruloyltyramine (2), *N*-trans-*p*-coumaroyltyramine (3), *N*-cis-*p*-coumaroyltyramine (4), *N*-trans-*p*-coumaroyloctopamine (5), *N*-cis-*p*-coumaroyloctopamine (6), ferulic acid (7), apigenin (8), luteolin (9), caffeic acid (10), 5-oxoaporphine (11), and oleanolic acid (12). Among these, compounds 2 and 4–9 were isolated from the genus *Sabia* for the first time, while compounds 1, 3, and 10 were reported from this plant species for the first time.

Keywords: *Sabia parviflora*; chemical constituents; amide alkaloids; apigenin; 5-oxoaporphine

Sabia parviflora Wall. ex Roxb. belongs to the family Sabiaceae and genus *Sabia*. The dried stems and leaves of this plant have a bitter taste and slightly cold properties, representing one of the most commonly used medicines among the Miao and Buyi ethnic groups in Guizhou for treating hepatitis and rheumatism. It possesses heat-clearing, dampness-removing, and hemostatic effects. The plant is mainly distributed in Guizhou, Guangxi, Sichuan, and Yunnan provinces at altitudes of 800–2800 m. According to literature reports (Wen et al., 2016; Zhao et al., 2018), the main chemical components in *S. parviflora* include alkaloids, pentacyclic triterpenoids, flavonoids, and phenylpropanoids.

In this study, the chemical constituents of the methanol ultrasonic extract from dried leaves of *S. parviflora* were systematically investigated using repeated silica gel column chromatography, Sephadex LH-20 column chromatography, preparative thin-layer chromatography, and recrystallization. Twelve compounds were isolated and their structures were elucidated based on physicochemical properties and spectroscopic analysis. These were identified as *N*-trans-feruloyltyramine (1), *N*-cis-feruloyltyramine (2), *N*-trans-*p*-coumaroyltyramine (3), *N*-cis-*p*-coumaroyltyramine (4), *N*-trans-*p*-coumaroyloctopamine (5), *N*-cis-*p*-coumaroyloctopamine (6), ferulic acid (7), apigenin (8), luteolin (9), caffeic acid (10), 5-oxoaporphine (11), and oleanolic acid (12). Among these, compounds 2 and 4–9 are reported from the genus *Sabia* for the first time, while compounds 1, 3, and 10 are reported from this plant for the first time.

1.1 Instruments and Materials

Instruments: Bruker AVANCE-500 NMR spectrometer (Bruker, Switzerland); Finnigan Trace DSQ quadrupole mass spectrometer (Thermo, USA); rotary evaporator (IKA, Germany); preparative silica gel TLC F254 plates (Merck, Germany); Sephadex LH-20 (Amersham Pharmacia Biotech AB). All chemical reagents were of analytical grade.

Materials: The plant material was collected in Xiuwen County, Guizhou Province in April 2014 and identified as the leaves of *Sabia parviflora* Wall. ex Roxb. by Professor SUN Qing-Wen from Guiyang College of Traditional Chinese Medicine. A voucher specimen (No. 20140426011) is deposited at the herbarium of Guiyang College of Traditional Chinese Medicine.

1.2 Extraction and Separation

Dried leaves of *S. parviflora* (1.5 kg) were extracted three times with methanol (3 L) under ultrasonic conditions (500 W) for 2 hours each time. The combined extracts were concentrated under reduced pressure to yield a crude extract (0.393 kg, yield 26.3%). The extract was suspended in water (1.0 L) and partitioned successively with equal volumes of *n*-hexane, ethyl acetate, and *n*-butanol, each three times. After concentration, *n*-hexane (46 g), ethyl acetate (93 g), and *n*-butanol (133 g) fractions were obtained.

The *n*-hexane fraction was subjected to silica gel column chromatography with a gradient elution of *n*-hexane-acetone to afford eight fractions (Fr. 1-8). Fraction 3 (3.1 g) was further separated by silica gel column chromatography using *n*-hexane-ethyl acetate gradient elution to give four subfractions (Fr. 3-1-3-4). Subfraction 3-2 (62.2 mg) was purified by Sephadex LH-20 column chromatography and preparative TLC to obtain compounds 1 and 2 (5.2 mg total), 3 and 4 (6.3 mg total), and 5 and 6 (7.2 mg total). Initially, compounds 1/2, 3/4, and 5/6 were obtained as mixtures in ratios of 2:1, 2:1, and 2.5:1, respectively, as estimated from their incompletely resolved ¹H NMR spectra. These three groups of cis-trans isomers were then separated by preparative silica gel TLC using *n*-hexane:ethyl acetate (1:2) as the developing solvent, yielding pure compounds 1 (2.8 mg), 2 (1.3 mg), 3 (3.8 mg), 4 (1.7 mg), 5 (4.7 mg), and 6 (1.9 mg). Subfraction 3-3 (25.2 mg) was purified by Sephadex LH-20 column chromatography and preparative TLC to afford compound 11 (15.1 mg).

The ethyl acetate fraction was subjected to silica gel column chromatography with a gradient elution of chloroform-methanol to give six fractions (Fr. 1-6). Fraction 3 (2.5 g) was further separated by silica gel column chromatography to yield seven subfractions (Fr. 3-1-3-7). Subfraction 3-2 (40.3 mg) was purified by Sephadex LH-20 column chromatography, preparative TLC, and recrystallization to obtain compounds 7 (15.1 mg), 8 (11.7 mg), 9 (9.5 mg), and 10 (19.3 mg). Subfraction 3-3 (34.7 mg) was purified by silica gel column chromatography, Sephadex LH-20 column chromatography, and recrystallization to afford compound 12 (24.2 mg).

The structures of compounds 1-12 are shown in Figure 1.

- 1: R =OCH , R =H
- 3: R =H, R =H
- 5: R =H, R =OH
- 2: R =OCH , R =H
- 4: R =H, R =H
- 6: R =H, R =OH

[Figure 1: see original paper] Chemical structures of compounds 1-12

2 Structure Identification

Compound 1: White powder. EI-MS m/z : 313 [M]. ^1H NMR (500 MHz, DMSO- d) : 7.27 (1H, d, J = 15.5 Hz, H-7), 7.08 (1H, d, J = 1.5 Hz, H-2), 7.02 (2H, d, J = 8.5 Hz, H-2, 6), 7.00 (1H, dd, J = 1.5, 8.0 Hz, H-6), 6.74 (1H, d, J = 8.0 Hz, H-5), 6.66 (2H, d, J = 8.5 Hz, H-3, 5), 6.38 (1H, d, J = 15.5 Hz, H-8), 3.79 (3H, s, 3-OCH), 3.26 (2H, t, J = 7.0, 14.0 Hz, H-8), 2.62 (2H, t, J = 7.0 Hz, H-7). ^{13}C NMR (125 MHz, DMSO- d) : 126.9 (C-1), 121.1 (C-2), 148.3 (C-3), 147.4 (C-4), 115.7 (C-5), 124.1 (C-6), 138.9 (C-7), 119.0 (C-8), 165.3 (C-9), 129.6 (C-1), 129.5 (C-2, 6), 115.1 (C-3, 5), 155.7 (C-4), 34.3 (C-7), 40.6 (C-8), 55.5 (OCH). These data are consistent with literature values (Yin et al., 2013), and thus compound 1 was identified as *N*-trans-feruloyltyramine.

Compound 2: White powder. EI-MS m/z : 313 [M]. ^1H NMR (500 MHz, DMSO- d) : 7.08 (1H, d, J = 1.5 Hz, H-2), 7.02 (2H, d, J = 8.0 Hz, H-2, 6), 6.96 (1H, dd, J = 1.5, 8.5 Hz, H-6), 6.70 (1H, d, J = 8.5 Hz, H-5), 6.65 (2H, d, J = 8.0 Hz, H-3, 5), 6.47 (1H, d, J = 13.0 Hz, H-7), 5.77 (1H, d, J = 13.0 Hz, H-8), 3.79 (3H, s, 3-OCH), 3.26 (2H, t, J = 7.5 Hz, H-8), 2.62 (2H, t, J = 7.5 Hz, H-7). ^{13}C NMR (125 MHz, DMSO- d) : 126.9 (C-1), 121.1 (C-2), 147.4 (C-3), 146.9 (C-4), 114.9 (C-5), 124.1 (C-6), 136.9 (C-7), 114.3 (C-8), 166.3 (C-9), 129.6 (C-1), 129.5 (C-2, 6), 115.2 (C-3, 5), 155.7 (C-4), 34.3 (C-7), 40.6 (C-8), 55.5 (OCH). These data are consistent with literature values (Yin et al., 2013), and thus compound 2 was identified as *N*-cis-feruloyltyramine.

Compound 3: White powder. EI-MS m/z : 283 [M]. ^1H NMR (500 MHz, DMSO- d) : 7.37 (2H, d, J = 8.5 Hz, H-2, 6), 7.30 (1H, d, J = 15.5 Hz, H-7), 7.01 (2H, d, J = 8.0 Hz, H-2, 6), 6.78 (2H, d, J = 8.5 Hz, H-3, 5), 6.69 (2H, d, J = 8.0 Hz, H-3, 5), 6.38 (1H, d, J = 15.5 Hz, H-8), 3.26 (2H, d, J = 7.5 Hz, H-8), 2.61 (2H, d, J = 7.5 Hz, H-7). ^{13}C NMR (125 MHz, DMSO- d) : 126.7 (C-1), 129.9 (C-2), 115.6 (C-3), 159.5 (C-4), 115.6 (C-5), 129.9 (C-6), 139.0 (C-7), 119.1 (C-8), 165.8 (C-9), 126.2 (C-1), 129.6 (C-2, 6), 115.2 (C-3, 5), 156.1 (C-4), 34.9 (C-7), 41.1 (C-8). These data are consistent with literature values (Wei and Lou, 2012), and thus compound 3 was identified as *N*-trans-*p*-coumaroyltyramine.

Compound 4: White powder. EI-MS m/z : 283 [M]. ^1H NMR (500 MHz, DMSO- d) : 7.59 (2H, d, J = 8.3 Hz, H-2, 6), 7.00 (2H, d, J = 8.2 Hz, H-2,

6), 6.70 (2H, d, $J = 8.3$ Hz, H-3, 5), 6.67 (2H, d, $J = 8.2$ Hz, H-3, 5), 6.48 (1H, d, $J = 13.0$ Hz, H-7), 5.75 (1H, d, $J = 13.0$ Hz, H-8), 3.31 (2H, d, $J = 7.4$ Hz, H-8), 2.64 (2H, d, $J = 7.4$ Hz, H-7). ^{13}C NMR (125 MHz, DMSO- d) : 126.7 (C-1), 130.0 (C-2), 115.6 (C-3), 158.4 (C-4), 115.6 (C-5), 130.0 (C-6), 139.0 (C-7), 119.1 (C-8), 166.6 (C-9), 126.2 (C-1), 129.6 (C-2, 6), 115.2 (C-3, 5), 156.1 (C-4), 34.7 (C-7), 40.9 (C-8). These data are consistent with literature values (Wei and Lou, 2012), and thus compound 4 was identified as *N*-cis-*p*-coumaroyltyramine.

Compound 5: White powder. EI-MS m/z : 299 [M]. ^1H NMR (500 MHz, DMSO- d) : 7.35 (2H, d, $J = 8.5$ Hz, H-2, 6), 7.29 (1H, d, $J = 15.6$ Hz, H-7), 7.13 (2H, d, $J = 8.5$ Hz, H-2, 6), 6.76 (2H, d, $J = 8.5$ Hz, H-3, 5), 6.71 (2H, d, $J = 8.5$ Hz, H-3, 5), 6.47 (1H, d, $J = 15.6$ Hz, H-8), 4.53 (1H, m, H-7), 3.17 (2H, m, H-8 a/8 b). ^{13}C NMR (125 MHz, DMSO- d) : 125.7 (C-1), 129.6 (C-2, 6), 116.3 (C-3, 5), 158.7 (C-4), 139.2 (C-7), 118.8 (C-8), 166.1 (C-9), 134.5 (C-1), 127.6 (C-2, 6), 115.2 (C-3, 5), 156.9 (C-4), 71.6 (C-7), 47.5 (C-8). These data are consistent with literature values (Wei and Lou, 2012), and thus compound 5 was identified as *N*-trans-*p*-coumaroyloctopamine.

Compound 6: White powder. EI-MS m/z : 299 [M]. ^1H NMR (500 MHz, DMSO- d) : 7.62 (2H, d, $J = 8.5$ Hz, H-2, 6), 7.12 (2H, d, $J = 8.5$ Hz, H-2, 6), 6.70 (2H, d, $J = 8.5$ Hz, H-3, 5), 6.69 (2H, d, $J = 8.5$ Hz, H-3, 5), 6.47 (1H, d, $J = 13.0$ Hz, H-7), 5.78 (1H, d, $J = 13.0$ Hz, H-8), 4.53 (1H, m, H-7), 3.17 (2H, m, H-8 a/8 b). ^{13}C NMR (125 MHz, DMSO- d) : 125.7 (C-1), 129.6 (C-2, 6), 116.3 (C-3, 5), 158.7 (C-4), 137.5 (C-7), 118.9 (C-8), 166.6 (C-9), 134.5 (C-1), 127.6 (C-2, 6), 115.2 (C-3, 5), 156.9 (C-4), 71.5 (C-7), 47.4 (C-8). These data are consistent with literature values (Wei and Lou, 2012), and thus compound 6 was identified as *N*-cis-*p*-coumaroyloctopamine.

Compound 7: Light yellow crystals. EI-MS m/z : 194 [M]. ^1H NMR (500 MHz, CDCl $_3$) : 7.21 (1H, d, $J = 2.0$ Hz, H-2), 6.78 (1H, d, $J = 8.0$ Hz, H-5), 7.08 (1H, dd, $J = 8.0, 2.0$ Hz, H-6), 7.51 (1H, d, $J = 16.0$ Hz, H-7), 6.39 (1H, d, $J = 16.0$ Hz, H-8), 3.91 (3H, -OCH $_3$). ^{13}C NMR (125 MHz, CDCl $_3$) : 127.2 (C-1), 109.4 (C-2), 146.5 (C-3), 147.7 (C-4), 114.0 (C-5), 123.5 (C-6), 56.1 (-OCH $_3$). These data are consistent with literature values (Wang et al., 2014), and thus compound 7 was identified as ferulic acid.

Compound 8: Light yellow powder. EI-MS m/z : 270 [M]. ^1H NMR (500 MHz, DMSO- d) : 12.96 (1H, s, 5-OH), 7.92 (2H, d, $J = 9.0$ Hz, H-2, 6), 6.92 (2H, d, $J = 9.0$ Hz, H-3, 5), 6.78 (1H, s, H-3), 6.48 (1H, d, $J = 2.1$ Hz, H-8), 6.18 (1H, d, $J = 2.1$ Hz, H-6). ^{13}C NMR (125 MHz, DMSO- d) : 94.0 (C-8), 98.8 (C-6), 102.8 (C-3), 103.7 (C-10), 116.0 (C-3, 5), 121.2 (C-1), 128.5 (C-2, 6), 157.3 (C-9), 161.2 (C-4), 161.4 (C-5), 163.7 (C-7), 164.2 (C-2), 181.7 (C-4). These data are consistent with literature values (Xu, 2005; Tian et al., 2005), and thus compound 8 was identified as apigenin.

Compound 9: Yellow powder. EI-MS m/z : 286 [M]. ^1H NMR (500 MHz, DMSO- d) : 12.97 (1H, s, 5-OH), 10.87 (1H, s, 7-OH), 9.96 (1H, s, 4-OH), 9.45

(1H, s, 3-OH), 7.42 (1H, dd, $J = 8.3, 2.2$ Hz, H-6), 7.39 (1H, d, $J = 2.2$ Hz, H-2), 6.89 (1H, d, $J = 8.3$ Hz, H-5), 6.67 (1H, s, H-3), 6.44 (1H, d, $J = 2.0$ Hz, H-8), 6.19 (1H, d, $J = 2.0$ Hz, H-6). ^{13}C NMR (125 MHz, DMSO- d) : 164.6 (C-2), 103.3 (C-3), 182.1 (C-4), 161.9 (C-5), 99.3 (C-6), 164.6 (C-7), 94.3 (C-8), 157.8 (C-9), 104.2 (C-10), 122.0 (C-1), 113.8 (C-2), 146.2 (C-3), 150.2 (C-4), 116.5 (C-5), 119.5 (C-6). These data are essentially consistent with literature values (Xu and Liang, 2005), and thus compound 9 was identified as luteolin.

Compound 10: Light yellow powder. EI-MS m/z : 180 [M]. ^1H NMR (500 MHz, CD OD) : 6.20 (1H, d, $J = 16.0$ Hz, H-8), 6.79 (1H, d, $J = 8.5$ Hz, H-5), 6.95 (1H, dd, $J = 8.5, 2.0$ Hz, H-6), 7.02 (1H, d, $J = 2.0$ Hz, H-2), 7.53 (1H, d, $J = 16.0$ Hz, H-7). ^{13}C NMR (125 MHz, CD OD) : 127.7 (C-1), 115.8 (C-2), 146.9 (C-3), 149.4 (C-4), 116.6 (C-5), 122.8 (C-6), 147.0 (C-7), 115.1 (C-8), 171.1 (C-9). These data are essentially consistent with literature values (Zhu et al., 2017), and thus compound 10 was identified as caffeic acid.

Compound 11: Yellow needle-like crystals. EI-MS m/z : 279 [M]. ^1H NMR (500 MHz, DMSO- d) : 2.68 (1H, t, $J = 14.2$ Hz, H-7), 3.12 (1H, dd, $J = 14.6, 5.2$ Hz, H-7), 3.29 (1H, m, H-4), 4.54 (1H, d, $J = 13.8$ Hz, H-6a), 6.04, 6.18 (each 2H, s, OCH O), 6.76 (1H, s, H-3), 7.26-7.36 (3H, m, H-8, 9, 10), 8.00 (1H, d, $J = 7.7$ Hz, H-11), 8.27 (1H, s, -NH). ^{13}C NMR (125 MHz, DMSO- d) : 142.6 (C-1), 147.4 (C-2), 106.6 (C-3), 124.6 (C-3a), 36.2 (C-4), 168.8 (C-5), 49.9 (C-6a), 35.6 (C-7), 133.8 (C-7a), 128.5 (C-8), 128.0 (C-9), 127.3 (C-10), 126.4 (C-11), 130.3 (C-11a), 114.8 (C-11b), 124.0 (C-11c), 101.1 (OCH O). These data are consistent with literature values (Liu et al., 2014), and thus compound 11 was identified as 5-oxoaporphine.

Compound 12: White needle-like crystals. EI-MS m/z : 456 [M]. ^1H NMR (500 MHz, CDCl) : 0.75, 0.77, 0.90, 0.91, 0.93, 0.98, 1.13 (each 3H, s, $7\times\text{CH}$), 5.30 (1H, brs, H-12). ^{13}C NMR (125 MHz, CDCl) : 38.6 (C-1), 27.5 (C-2), 79.2 (C-3), 38.9 (C-4), 55.4 (C-5), 18.5 (C-6), 32.8 (C-7), 39.4 (C-8), 47.8 (C-9), 37.2 (C-10), 23.2 (C-11), 122.8 (C-12), 143.8 (C-13), 41.8 (C-14), 27.8 (C-15), 23.6 (C-16), 46.7 (C-17), 41.1 (C-18), 46.0 (C-19), 30.8 (C-20), 34.0 (C-21), 32.6 (C-22), 28.2 (C-23), 15.8 (C-24), 15.6 (C-25), 17.3 (C-26), 26.1 (C-27), 183.5 (C-28), 32.2 (C-29), 23.7 (C-30). These data are essentially consistent with literature values (Liu et al., 2014), and thus compound 12 was identified as oleanolic acid.

In this study, twelve compounds were isolated from the dried leaves of *S. parviflora*. Among them, compounds 2 and 4-9 are reported from the genus *Sabia* for the first time, while compounds 1, 3, and 10 are reported from this plant for the first time. Compounds 1-6 are phenylpropanoid amide alkaloids, compounds 8-9 are flavonoids, and compounds 7 and 10 are phenylpropanoids. Additionally, two common compounds from *S. parviflora* were obtained: compound 11 is an aporphine alkaloid, and compound 12 is a pentacyclic triterpenoid saponin. These compound types are fully consistent with previously reported chemical constituents from *S. parviflora*. From a structural perspective, the isolation of six phenylpropanoid amide alkaloids suggests that this class of compounds is relatively common in *S. parviflora*, and other alkaloids with structures similar

to compounds 1-6 should also be widely distributed in this plant.

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