Bioactivity-guided isolation of compounds from *Sophora flavescens* with antibacterial activity against *Acinetobacter baumannii*

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**ABSTRACT**

Bioactivity-guided fraction of an extract of *Sophora flavescens* to identify antibacterial compounds against *Acinetobacter baumannii*, led to the isolation of two new compounds, (2'SR)-5-methoxy-7-hydroxy-8-lavandulylchromone (13) and (2'S,βS)-(-)-sophobiflavonoid CE (19), and 18 known flavonoids, (6aR,11aR)(-)-maackiain (1), (25)(-)-8-prenylnaringenin (2), (25)(-)-exiguafлаванон K (3), (25)(-)-sophoraflavanone G (4), (25)(-)-leachianone A (5), (25)(-)-kushenol E (6), (25)(-)-leachianone G (7), (α)-kushenol F (8), (25)(-)-kurarinone (9), (25)(-)-kurarisol (10), (2R,3R)-(+)-3,7,4'-trihydroxy-5-methoxy-8-prenylflavanone (11), (25)(-)-isonaxthohumol (12), (25)(-)-2'-methoxykurarinone (14), (2R,3R)(+)-kushenol I (15), calycosin (16), kurarin (17), (25)(-)-kushenol A (18), and trifolirhizin (20). Their structures were elucidated based on NMR, MS, and CD spectroscopic analysis. Among them, 1, 2, 5, and 15 exerted modest antibacterial activity against *A. baumannii*, with MIC95 of 128-256 µg/mL for 2 and 256-512 µg/mL for 1, 5 and 15.

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1. Introduction

*Acinetobacter baumannii* is a Gram-negative bacterium and an opportunistic human pathogen, which can cause a broad range of infections, including respiratory tract infections, pneumonia, septicaemia, infections of the skin and soft tissues, and urinary tract infections. It is becoming increasingly important as a cause of hospital-acquired infections (Harding et al. 2018). Carbapenem-resistant *A. baumannii* is at the top of the World Health Organization’s list of resistant pathogens for which new antimicrobial discovery is a critical need (Willyard 2017). Plants have been used as remedies in traditional medicine for thousands of years, yet compounds from these plants are still an underutilized source of antimicrobials.

*Sophora flavescens* Ait. (Fabaceae) is a medicinal plant distributed mainly in China, Korea and Japan (Huang et al. 2017). The dried root of *S. flavescens* (Chinese name “KuShen”) has heat-clearing and damp-drying functions in traditional medicine. It has been used in China and some other Asian countries for thousands of years in the treatment of fever, diarrhea, jaundice, eczema, vaginal itching with leukorrhagia, anur-esis, gastrointestinal hemorrhage, inflammatory disorders, and microbial infections (Chinese Pharmacopoeia Commission 2015; He et al. 2015). Flavonoids and alkaloids are the two principal chemical classes in *S. flavescens* (He et al. 2015). Du et al. (2010) found that the total flavonoid extract from *S. flavescens* was more potent than the total alkaloid extract in inhibiting Gram-positive bacteria including *Staphylococcus* species and Gram-negative bacteria including *Pseudomonas aeruginosa*, *Escherichia coli* and *Shigella flexneri*. Huang et al. (2016) also demonstrated that the total flavonoid extract was more potent than the total alkaloid extract against *S. aureus* and *E. coli*, with MICs of 0.001-0.004 mg/L and 0.001-0.004 mg/L, respectively for the flavonoid extract and 0.005-0.032 mg/L and 0.008-0.032 mg/L, respectively for the alkaloid extract. Some flavonoids isolated from *S. flavescens* have been reported to inhibit *S. aureus*, *B. subtilis*, *S. epidermidis*, *E. coli*, *Propionibacterium acnes*, and *Salmonella typhi-murium* (Kuroyanagi et al. 1999; Sohn et al. 2004; Huang et al. 2016).

In our previous anti-bacterial screening of 50 traditional Chinese medicines, it was first found that an 80% ethanolic ultrasonic extract of *S. flavescens* displayed anti-bacterial activity against *A. baumannii*, with a MIC$_{95}$ value of 512 µg/mL (Figure S1). While this activity was only weak, we hypothesized that individual compounds within this extract may display improved activity against this critical priority pathogen. In this study, we undertook bioactivity-guided isolation of the compounds from *S. flavescens* and testing of purified compounds against *A. baumannii*.

2. Results and discussion

2.1. Bioactivity-guided fraction of the 80% ethanol extract of *S. flavescens*

The bioactivity-guided fraction of the 80% ethanol extract of *S. flavescens* (Figure S1) showed that both the 80% ethanol extract (SF1) and its water-insoluble fraction (SF-3) dose-dependently inhibited *A. baumannii*, with 82.9 ± 3.4% and 80.2 ± 5.3% inhibition at 512 µg/mL. Further column chromatography showed that the fraction eluted with EtOAc (SF5) was the most active fraction in SF3. Further fractionation of SF5 afforded...
9 sub-fractions. As shown in Figure S2, SF5-(23-35) showed some anti-bacterial activity (MIC$_{95}$ of 256-512 µg/mL), and the other eight fractions showed partial inhibitory effects, with inhibition rates ranging from 40.7-76.2% at 512 µg/mL.

### 2.2. Isolation and structural elucidation of the compounds

Further repeated column chromatography of above fractions afforded a total of 20 compounds. 1 was isolated from SF5-(13-22), 1-6 were isolated from SF5-(23-35), 4, 7-8 were isolated from SF5-(36-44), 9-19 were isolated from SF5-(45-50), 9-10 were also isolated from SF5-(51-56), 20 was isolated from SF5-(57-59). Their structures were identified based on spectroscopic data (Supporting information). Among them, 5-methoxy-7-hydroxy-8-lavandulylchromone (13) and (2S,βS)-(−)-sophorafлавonoid CE (19) (Yan et al. 2019) were new compounds, and the 18 known compounds were (6αR,11αR)-(-)-maackiain (1) (Wang et al. 2014; Selvam et al. 2017), (2S,βS)-(−)-8-prenylnaringenin (2) (Mizobuchi and Sato 1984), (2S)-(−)-exiguafлавanone K (3) (lumina et al. 1994), (2S)-(−)-sophorаflavanone G (4) (Shirataki et al. 1988), (2S)-leachianone A (5) (Hillerns and Wink 2005), (2S)-(−)-kushenol E (6) (Li et al. 2008a), (2S)-(−)-leachianone G (7) (lumina...
Compound 13 was a white amorphous powder. It showed a dark-blue color when sprayed with the FeCl₃ reagent, indicating that 13 was a phenolic compound. Its molecular formula was established as C₂₀H₂₄O₄ according to the HR-ESI-MS pseudomolecular ion peak at m/z 329.1752 [M + H]+ (calcd. for C₂₀H₂₅O₄, 329.1753). The ¹H-NMR spectrum (600 MHz, Acetone-d₆) of 13 showed an aromatic hydroxyl proton at δₜ 9.30 (1H, s), a pair of cis-alkene hydrogens at δₜ 7.89 (1H, d, J = 6.0 Hz, H-2) and 6.00 (1H, d, J = 6.0 Hz, H-3), a methoxyl group at δₜ 3.78 (3H, s), an isolated aromatic proton at δₜ 6.49 (1H, s), and a characteristic lavandulyl group at δₜ 1.55 (3H, s, H-7”), 1.62 (3H, s, H-6”), 1.72 (3H, s, H-10”), 2.15 (2H, m, H-3”), 2.54 (1H, m, H-2”), 2.81 (2H, m, H-1”), 4.58 (1H, dd, J = 2.4, 1.2 Hz, H-9”a), 4.49 (1H, d, J = 2.4 Hz, H-9”b) and 5.06 (1H, t, J = 7.2 Hz, H-4”). The ¹³C-NMR (150 MHz, Acetone-d₆) and HMBC spectra showed 13 contained twenty carbons, including a lavandulyl group, a methoxy carbon (δC 55.3), a carbonyl group (δC 175.3), an oxygenated cis-alkene group (δC 152.7 and 113.6), and a benzene ring. These data indicated 13 was a benzopyranone (i.e., chromone) possessing lavandulyl, methoxyl, and hydroxyl moieties. The HMBC spectra (Figure S3, Supporting information) indicated that the oxygenated cis-alkene proton (δₜ 7.89) correlated with the alkene carbon at δC 113.6 (C-3), oxygenated aromatic carbon at δC 157.8 (C-9) and carbonyl carbon at δC 175.3 (C-4). Another cis-alkene proton (δₜ 6.00) correlated with aromatic carbon at δC 109.3 (C-10) and oxygenated alkene carbon at δC 152.7 (C-2), confirming the presence of a chromone. In addition, H-1” (δₜ 2.82) of the lavandulyl group correlated with δC 158.9 (C-7), 107.5 (C-8) and 157.8 (C-9), the methoxyl proton (δC 3.78) correlated with δC 159.7 (C-5), and the isolated aromatic proton (δₜ 6.49) correlated with δC 158.9 (C-7), 159.7 (C-5), 107.5 (C-8) and 109.3 (C-10), indicating the lavandulyl, the hydroxyl and the methoxyl groups are located at C-8, C-7 and C-5, respectively. Compared with (–)-R- and (+)-S-lavandulol (Fernandes and Chowdhury 2013), 13 possessing only one lavandulyl moiety and a levorotation ([α]20 D −5.6 (c 0.1, MeOH)) was deduced to be 2'R-configuration. Thus, 13 was elucidated as (2’R)-5-methoxy-7-hydroxy-8-lavandulyl-chromone.

Compound 19 was a yellow amorphous powder. It showed a dark-blue color when sprayed with the FeCl₃ reagent. The molecular formula of 19 was established as C₅₂H₆₀O₁₂ based on the HR-ESI-MS pseudomolecular ions at m/z 877.4169 [M + H]+ (calcd. for C₅₂H₆₁O₁₂, 877.4163) and m/z 899.3981 [M + Na]+ (calcd. for C₅₂H₆₀NaO₁₂, 899.3982). Its ¹H-NMR spectrum (600 MHz, Acetone-d₆) indicated diagnostic flavanone signals at δₜ 5.58 (1H, dd, J = 13.2, 1.8 Hz, H-2), 2.77 (1H, dd, J = 16.2, 13.2 Hz, H-3) and 2.54 (1H, dd, J = 16.2, 1.8 Hz, H-3), an ABX spin system including aromatic protons at δₜ 7.00 (1H, d, J = 8.4 Hz, H-6”), 6.38 (1H, d, J = 2.4 Hz, H-3”) and 6.27 (1H, dd, J = 8.4, 2.4 Hz, H-5”), four single aromatic protons at δₜ 7.52 (1H, s, H-6’), 6.44 (1H, s, H-3’), 6.19 (1H, s, H-6) and 6.06 (1H, s, H-6”), a methane proton at δₜ 5.25 (1H, t, J = 7.0 Hz, H-β), two methylene protons at δₜ 3.97 (1H, dd, J = 17.4, 7.2 Hz, H-α₁) and 3.74 (1H, overlapped, H-α₂), two methoxy groups at δₜ 3.74 (3H, s, 5-OCH₃) and 3.83 (3H, s, 5”-
OCH₃) and two sets of lavandulyl groups. The ¹³C-NMR (150 MHz, Acetone-d₆) of 19 contained 52 carbons, including 15 flavanone skeleton carbons, 15 dihydrochalcone skeleton carbons, 2 methoxy carbons and 20 carbons belonging to two lavandulyl groups, suggesting 19 was a biflavonoid composed of one flavanone and one dihydrochalcone. The HMBC spectrum showed that δ_H 5.25 (H-β) correlated with δ_C 48.0 (C-α), 204.0 (C-4”), 155.0 (C-4’), 122.6 (C-5’), 126.1 (C-6’), 122.6 (C-1”’), 154.7 (C-2”’), and 128.6 (C-6”’), indicating the flavanone and dihydrochalcone are linked through a C5’-Cβ bond. Moreover, δ_H 2.68 (H-1a) had HMBC correlations with δ_C 161.5 (C-7), 107.8 (C-8), 163.0 (C-9), and δ_H 2.61 (H-1a”) had HMBC correlations with δ_C 162.3 (C-7”), 107.3 (C-8”), 165.3 (C-9”), indicating that the two lavandulyl groups are connected with C-8 and C-8”, respectively. The HMBC spectrum also showed that δ_H 3.74 (5-OCH₃) and 3.84 (5”-OCH₃) correlated with δ_C 160.3 and 161.2, and aromatic protons δ_H 6.19 (H-6) and δ 6.06 (H-6”) correlated with δ_C 160.3 (C-5), 161.5 (C-7), 107.8 (C-8), 163.0 (C-9), 105.3 (C-10), and 161.2 (C-5”), 162.3 (C-7”), 107.3 (C-8”), 105.2 (C-10”), respectively, indicating the two methoxy groups are connected with C-5 and C-5”. The above data indicated that 19 had the same planar structure as sophobiflavonoid A, B, and C, recently isolated new biflavonoids (Yan et al. 2019). However, in contrast to the CD spectra of (2S,βR)-sophobiflavonoid A, (2R,βS)-sophobiflavonoid B, and (2R,βR)-sophobiflavonoid C (a negative Cotton effect at 337 nm, 226 nm and 204 nm, and a positive Cotton effect at 293 nm and 243 nm) (Yan et al. 2019), 19 showed a positive Cotton effect at 337 nm, 229 nm, and 205 nm, and a negative Cotton effect at 296 nm and 242 nm in its CD spectrum (Supporting information), which was the opposite of sophobiflavonoid C. Hence, 19 was assigned a 2S,βS configuration and named as sophobiflavonoid CE.

2.3. Anti-bacterial screening of the compounds against A. baumannii

Among the 20 compounds, maackiain (1), prenylnaringenin (2), leachianone A (5) and kushenol I (15) displayed the highest inhibition against A. baumannii with a dose-dependent effect (Figure S4), and an MIC₉₅ of 128-256 µg/mL for 2 and 256-512 µg/mL for 1, 5, and 15. Although MIC₉₅ of sophoraflavonane G (4) exceeded 512 µg/mL, it still displayed 45% inhibition at low dose of 8 µg/mL. When the equivalent hydroxyl group of maackiain (1) is linked to glucose, as in the glycoside trifolirhizin (20), the anti-bacterial activity completely disappears. Kurarione (9) was isolated in the largest quantity (0.02%) among the isolated compounds, however its antibacterial activity was weak.

3. Experimental

3.1. General

Optical rotations were determined on a Gyromat-Hp automatic digital polarimeter (Kernchen Co., Seelze, Germany). UV spectra were obtained with a UV-2550 UV-Vis spectrophotometer (Shimadzu Co., Kyoto, Japan). CD spectra were recorded on a Chirascan instrument (Applied Photophysics, Leatherhead, UK). IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer (Thermo Fisher Scientific, MA, USA). NMR spectra were measured on an Avance DRX-600 spectrometer (600 MHz for ¹H NMR and 150 MHz for ¹³C NMR, Bruker Company, Fläddalen, Switzerland) with TMS as the internal standard.
HR-ESI-MS was recorded on LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific, MA, USA). The ESI-MS was obtained with a single quadrupole LC-MS 2020 mass spectrometer (Shimadzu, Japan). Semi-preparative HPLC was performed on LC-20AT high-performance liquid chromatography instrument (Shimadzu Co., Kyoto, Japan) with a UV detector and a Hypersil ODS (YMC-Pack ODS-A, 250 mm × 10 mm, 5 μm, Tokyo, Japan). Silica gel (200-300 mesh; Qingdao Marine Chemical Plant, Qingdao, China), polyamide gel (100-200 mesh; Taizhou Luqiao Siqing Biochemical Plastics Factory, Taizhou, China), ODS-C18 (75 μm, YMC Co., Japan), and Sephadex LH-20 (Pharmacia Fine Chemicals, NJ, USA) were used for column chromatography (CC).

3.2. Plant material

The root pieces of *S. flavescens* were bought from Jianlian Pharmacy (Jinan, China), and identified by Prof. Lan Xiang (one author). A voucher specimen (No. 20171201) was deposited in the Institute of Pharmacognosy, School of Pharmaceutical Sciences, Shandong University.

3.3. Extraction and isolation

Root powders of *S. flavescens* (900 g) were ultrasonically extracted with 80% ethanol at room temperature. The evaporated extract (SF1) was ultrasonically re-extracted with distilled water, yielding a water-soluble fraction (SF2, 143.3 g) and a water-insoluble fraction (SF3, 37.43 g). The bioactive SF3 was dissolved in 95% ethanol and mixed with 160 g celatom, then eluted sequentially with petroleum ether, ethyl acetate, ethyl acetate: methanol (89:11), and methanol to afford four sub-fractions (SF4-SF7). The bioactive SF5 (11.45 g) was purified by silica gel CC (500 g, 100-200 mesh) and eluted with a gradient of petroleum ether-EtOAc (85:15 to 0:1, v/v) to afford ten subfractions (SF5-(13-22)~SF5-(60-64)).

SF5-(13-22) (40 mg) was purified by semi-preparative HPLC (60% MeOH in H2O, flow rate 0.8 mL min⁻¹) to afford 1 (22 mg). SF5-(23-35) (200 mg) was subjected to ODS-C₁₈ CC, eluted with MeOH-H₂O (40:60 to 100:0, v/v) and then purified by Sephadex LH-20 CC (MeOH) to afford 2 (9 mg), 3 (11 mg), 4 (2.5 mg), 5 (16.7 mg), and 6 (8.7 mg). SF5-(36-44) (476 mg) was purified by ODS-C₁₈ CC with a gradient of MeOH/H₂O (55:45 to 100:0, v/v) and Sephadex LH-20 CC (MeOH) to afford 7 (200 mg), 8 (1.5 mg), and 9 (6.3 mg). SF5-(45-50) (2.2573 g) was purified by polyamide CC (130 g, 100-200 mesh) and eluted with a gradient of EtOH-H₂O (30:70 to 100:0, v/v) to afford thirteen subfractions. Among them, fraction SF5-(45-50)-(38-46) (185 mg) was subjected to ODS-C₁₈ CC, eluted with MeOH-H₂O (30:70 to 100:0, v/v) and then separated by semi-preparative HPLC (MeOH/H₂O, with a ratio of 60:40, 70:30, 80:20, 91:9, respectively, 1.5 mL·min⁻¹) to give 10 (3.6 mg), 11 (5.8 mg), 12 (21.6 mg), 13 (7.5 mg), 14 (8.9 mg), 15 (1.85 mg). SF5-(45-50)-(47-73) (807 mg) was subjected to ODS-C₁₈ CC, eluted with a gradient of MeOH-H₂O (40:60 to 100:0, v/v) and Sephadex LH-20 CC (MeOH) to afford 16 (200 mg), 17 (7 mg), 18 (4 mg), 19 (7 mg), 20 (179-184) (258.3 mg) was subjected to polyamide CC (100-200 mesh) and Sephadex LH-20 CC (MeOH) to give 21 (23.3 mg). SF5-(51-56) (123 mg) was subjected to ODS-C₁₈ CC, eluted with MeOH-H₂O (40:60 to 100:0, v/v) and Sephadex
LH-20 CC (MeOH) to afford 9 (11 mg), 10 (2.4 mg). SF5-(57-59) (1.4 g) was subjected to polyamide CC (85 g, 100-200 mesh) using a gradient of MeOH/H2O (30:70 to 100:0, v/v) and silica gel CC (14 g, 200-300 mesh) to afford 20 (27.8 mg).

3.4. Spectral data

5-methoxy-7-hydroxy-8-lavandulyl-chromone (13): White powder; [α]20 D −5.6 (c 0.1, MeOH); 1H NMR (600 MHz, Acetone-d6) δH: 9.30 (1H, s, OH), 7.89 (1H, d, J = 6.0 Hz, H-2), 6.49 (1H, s, H-6), 6.00 (1H, d, J = 6.0 Hz, H-3), 5.06 (1H, t, J = 7.2 Hz, H-4"), 4.58 (1H, dd, J = 2.4, 1.2 Hz, H-9"a), 4.49 (1H, d, J = 2.4 Hz, H-9"b), 3.78 (3H, s, 5-OCH3), 2.81 (2H, m, H-1"), 2.54 (1H, m, H-2"), 2.15 (2H, m, H-3"), 1.72 (3H, s, H-10"), 1.62 (3H, s, H-6"), 1.55 (3H, s, H-7"); 13C NMR (150 MHz, Acetone-d6) δC: 175.3 (C-4), 159.7 (C-5), 158.9 (C-7), 157.8 (C-9), 152.7 (C-2), 147.8 (C-8"), 131.0 (C-5"), 123.3 (C-4"), 113.6 (C-3), 110.7 (C-9"), 109.3 (C-10), 107.5 (C-8), 95.9 (C-6), 55.3 (5-OCH3), 47.3 (C-2"), 31.2 (C-3"), 27.1 (C-1"), 25.0 (C-6"), 17.9 (C-10"), 17.0 (C-7"). HR-ESI-MS: m/z 329.1752 [M + H]+ (calcd. for C20H25O4, 329.1753).

(2S,βS)–(−)–Sophobiflavonoid CE (19): yellow amorphous powder; [α]20 D−9.8 (c 0.1, MeOH); UV (MeOH) λ max (log ε) 290 (4.46), 225 sh (4.65) nm; ECD (c 0.2 mg/mL, MeOH) λ max (Δε) 337 (+2.37), 296 (-5.39), 242 (-1.66), 229 (+5.53), 205 (+36.01) nm; 1H NMR (600 MHz, Acetone-d6) δH: 14.17 (1H, s, 9"-OH), 9.13 (1H, s, 7"-OH), 9.08 (1H, s, 7-OH), 8.33 (1H, s, 2'-OH), 7.95 (1H, s, 4"-OH), 7.52 (1H, s, H-6'), 7.00 (1H, d, J = 8.4 Hz, H-6'"), 6.44 (1H, s, H-3'), 6.38 (1H, d, J = 2.4 Hz, H-3'"), 6.27 (1H, dd, J = 8.4, 2.4 Hz, H-5'"), 6.19 (1H, s, H-6), 6.06 (1H, s, H-6"), 5.58 (1H, dd, J = 13.2, 1.8 Hz, H-2), 5.25 (1H, t, J = 7.2 Hz, H-β), 5.02 (1H, t, J = 6.6 Hz, H-4a), 4.96 (1H, t, J = 6.6 Hz, H-4a), 4.57 (1H, brs, H-9a), 4.55 (1H, brs, H-9a), 4.54 (1H, brs, H-9a), 4.51 (1H, brs, H-9a), 3.97 (1H, dd, J = 17.4, 7.2 Hz, H-2'), 3.84 (3H, s, 5"-OCH3), 3.74 (3H, s, 5-OCH3), 3.74 (1H, overlapped, H-3'), 2.77 (1H, dd, J = 16.2, 13.2 Hz, H-3), 2.68 (2H, m, H-1a), 2.61 (2H, m, H-1a'), 2.54 (1H, dd, J = 16.2, 1.8 Hz, H-3), 2.54 (1H, m, H-2a), 2.54 (1H, m, H-2a'), 2.15 (2H, m, H-3a), 2.05 (2H, m, H-3a', overlapped), 1.67 (3H, s, H-10a), 1.63 (3H, s, H-10a), 1.60 (3H, s, H-6a), 1.52 (3H, s, H-7a), 1.50 (3H, s, H-6a), 1.44 (3H, s, H-7a); 13C NMR (150 MHz, Acetone-d6) δC: 204.0 (C-4"), 188.7 (C-4), 165.3 (C-9"), 163.0 (C-9), 162.3 (C-7"), 161.5 (C-7), 161.2 (C-5"), 160.3 (C-5), 156.4 (C-4"), 155.0 (C-4'), 154.7 (C-2'"), 153.0 (C-2'), 148.3 (C-8a), 148.2 (C-8a), 130.6 (C-5a), 130.5 (C-5a), 128.6 (C-6'"), 126.0 (C-6'), 123.6 (C-4a), 123.7 (C-4a'), 122.6 (C-5', C-1'"), 117.2 (C-1'), 110.4 (C-9a'), 110.3 (C-9a), 107.8 (C-8), 107.3 (C-8"), 107.1 (C-5'"), 105.4 (C-10), 105.2 (C-10"), 102.8 (C-3'), 102.7 (C-3"'), 92.6 (C-6), 90.2 (C-6"), 74.2 (C-2), 48.0 (C-2'), 46.7 (C-2a), 46.6 (C-2a'), 44.8 (C-3), 31.7 (C-β), 31.1 (C-3a), 30.9 (C-3a), 27.3 (C-1a), 26.8 (C-1a'), 25.0 (C-6a'), 24.9 (C-6a), 18.4 (C-10a'), 18.1 (C-10a), 17.0 (C-7a, C-7a'); HR-ESI-MS: m/z 877.4169 [M + H]+ (calcd. for C52H61O12, 877.4163), 899.3981 [M + Na]+ (calcd. for C52H60NaO12, 899.3982).

3.5. Anti-bacterial screening

Extracts, fractions and compounds from S. flavescens were tested for antibacterial activity against A. baumannii ATCC 19606, which was obtained from stock cultures in glycerol broth preserved at −80 °C at University of South Australia, Clinical and Health Sciences, Adelaide, South Australia. Cation adjusted MH II broth (BD Australia) was used for
experiments to determine the minimum inhibitory concentration (MIC), following the protocol of the Clinical and Laboratory Standards Institute (2006) with slight modifications. The OD$_{630}$ was measured at time zero using a plate reader (BioTek). Plates were incubated overnight at 37°C for 20 ± 2 h in air then the OD$_{630}$ was again recorded. Then MIC was determined as the lowest concentration at which no visual growth was observed in the duplicate wells and for which there was at least 95% inhibition as measured by the change in absorbance compared to the no treatment control.

4. Conclusion

Bioactivity-guided fraction of anti-bacterial components from *S. flavescens* against *A. baumannii* led to the isolation of two new constituents (13, 19) and 18 known flavonoids, and discovery of four anti-bacterial flavonoids maackiain (1), 8-prenylnaringenin (2), leachianone A (5) and kushenol I (15). This study provides evidence contributing to the scientific rationale for the traditional uses of *S. flavescens* and its future development as natural anti-infectious agent.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Supporting information**

ESI-MS, UV, CD, 1D NMR and 2D NMR data were present in supporting information.

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