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Sesbagrandiflorain A and B: isolation of two new 2-arylbenzofurans from the stem bark of *Sesbania grandiflora*

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ABSTRACT

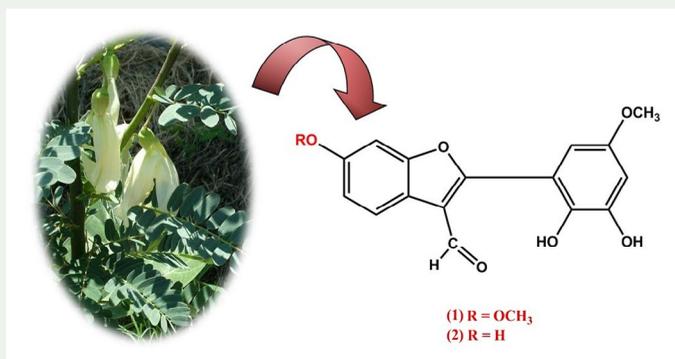
Native to tropical Asia, *Sesbania grandiflora* (L.), Pers is a member of the Fabaceae family of flowering plants. All parts of *S. grandiflora* are used in traditional medicine and phytochemical investigations have been conducted on extracts of the leaves, seeds and roots of *S. grandiflora* to provide scientific validation of its properties. However, to date, no study has determined the phytochemical constituents of *S. grandiflora* stem bark. The stem bark powdered of *S. grandiflora* was extracted exhaustively with *n*-hexane, EtOAc and 90% aqueous MeOH sequentially. In this study, we successfully isolated two new 2-arylbenzofurans, sesbagrandiflorain A and B, from the EtOAc stem bark of *S. grandiflora*. The structure elucidation of these compounds was determined by using one- and two-dimensional nuclear magnetic resonance, ultraviolet and infrared spectroscopy and electrospray ionisation time-of-flight mass spectrometry. The finding expands the understanding of the natural constituents of the Fabaceae and, in particular, the *Papilionoideae* genera.

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1. Introduction

Plants of the family Fabaceae, particularly species of the Papilionoideae subfamily, have been extensively investigated for their phytochemical and pharmacological properties (Gođevac et al. 2008). Several secondary metabolites have been isolated from members of this family, including alkaloids, non-protein amino acids, flavonoids, isoflavonoids, coumarins, phenylpropanoids, anthraquinones, terpenoids and cyanogenic glycosides (Wink and Mohamed 2003). Among them, isoflavonoids are predominantly found in plants of the Papilionoideae subfamily (Kırmızıbekmez et al. 2015). A large number of new and known isoflavonoids has been characterised from plants in which the majority of these compounds were isolated from the Fabaceae (Dewick 2005; Veitch 2007). Isoflavonoids, with their structural diversity, were reported to possess wide range of biological activities against different strains of bacteria, fungi, viruses, plasmodium and various cancer cell lines (Kraft et al. 2001; Lo et al. 2002; Koyosomboon et al. 2006).

Sesbania grandiflora is a member of the Fabaceae family native to tropical Asia, including India, Malaysia, Indonesia, Myanmar and the Philippines. All parts of *S. grandiflora* are used in traditional medicine particularly in south-eastern Asia and India to treat various diseases including bacterial infections. Generally, the root is applied as a poultice to relieve from inflammation and fever. Ground root of *S. grandiflora* var. *coccinea* mixed with water is applied externally as a poultice to treat rheumatic swellings. The bark is used as astringent to cure smallpox. In Philippines, the decoction from the crushed bark is used for the treatment of ulcers in the mouth and alimentary canal. In Java, the local healers use the crushed bark for the treatment of thrush and infantile disorders of the stomach, while in Cambodia, the pounded bark is applied to treat the scabies. The leaves juice is used to treat worms, biliousness, fever, gout, itchiness and leprosy. In Malaysia, the crushed leaves are applied to cure sprains and bruises. While in Ayurveda, the leaves are employed for the treatment of epileptic fits and the fruits are used for the treatment of anaemia, bronchitis, fever and tumours. The leaves and flowers juice is a popular remedy for nasal catarrh and headache, head congestion or stuffy nose. The flowers juice is dropped into the eyes to correct dim visions (Wagh et al. 2009).

Several studies have been conducted on extracts obtained from *S. grandiflora* trees (local name: turi) to provide scientific validation of its properties. The methylene chloride and methanol extracts of aerial parts of *S. grandiflora* have shown to have some antifungal activities (Goun et al. 2003). A subsequent research by Doddola et al. (2008) revealed that the leaf juice of *S. grandiflora* exhibits significant antiurolithiatic activity against calcium oxalate-type stones as well as antioxidant properties. Besides that, Laladhas et al. (2010) had evaluated the flowers of *S. grandiflora* *in vivo* and *in vitro* using different cancer cell lines, disclosed that the flowers may serve as a potential anticancer drug candidate. Moreover, the *n*-hexane extract of *S. grandiflora* seeds, which was investigated by Shareef et al. (2012), showed that it possessed antioxidant, anti-inflammatory, analgesic and antipyretic activities.

The earliest phytochemical investigations of *S. grandiflora* were conducted in the 1960s, and resulted in the isolation of α -5-methyl-5-pentacosanol for the first time from *S. grandiflora* leaves (Tiwari and Bajpai 1964). Recently, Pollard et al. (2011) reported the isolation of a galactomannan from the seeds of *S. grandiflora*. In addition, Hasan et al. (2012) and Noviany et al. (2012) obtained and evaluated the antituberculosis activity of several phenolic compounds from the roots of *S. grandiflora*.

Nowadays, the discovery of lead compounds for development of new scaffolds of drugs from plants, has received considerable attention. Based on the explanation above, *S. grandiflora* is one of the potential medicinal plants that can be employed as a source of new biologically active compounds. Hence, the phytochemical study on this plant is considered essential. Even though *S. grandiflora* was extensively studied by other researchers for its phytopharmacological potential, especially the leaves, flowers and aerial parts of the plant, no phytochemical studies extensively have been performed on the stem bark of *S. grandiflora*. In terms of finding the lead compounds therefore, in this study, we investigated the phytochemical constituents of the stem bark of *S. grandiflora*. The ethyl acetate (EtOAc) extract of *S. grandiflora* stem bark was subjected to further analysis, which led to the isolation of two new 2-arylbenzofurans.

2. Results and discussion

The EtOAc extract of *S. grandiflora* stem bark contained two compounds, **1** and **2** (structures are shown in Figure 1), after repeated Column Chromatography (CC) separation.

Sesbagrandiflorain A (Compound **1**) was obtained as a needle-like yellow crystal. The UV spectrum of Compound **1** in MeOH, with λ_{\max} at 213, 264, 348 and 363 nm, indicated the presence of a conjugated system in the isolated structure (Komatsu et al. 1981). The IR absorption of Compound **1** demonstrated the presence of a hydroxyl group (OH) at 3407 cm^{-1} , a saturated aliphatic carbon group (CH) at 2924 cm^{-1} , an aldehyde group (CHO) at 1652 cm^{-1} , and an olefinic group at 1438 cm^{-1} . ESI-TOF-MS of Compound **1** revealed a pseudomolecular ion peak $[M - H]^+$ at m/z 313.0709, which was consistent with a molecular formula of $C_{17}H_{14}O_6$. The structure elucidation of Compound **1** was deduced from detailed analysis of ^1H NMR and ^{13}C NMR spectral data aided by 2D NMR experiments such as ^1H - ^1H COSY, HSQC, HMBC and NOESY.

The ^1H NMR spectrum of Compound **1** (Table S1) revealed a set of aromatic proton resonances with an AMX splitting pattern of the A-ring at δ_{H} 6.67 (dd, $J = 8.4; 2.2$ Hz, H-5), 6.71 (d, $J = 2.2$ Hz, H-7) and 7.55 (d, $J = 8.4$ Hz, H-4), as well as a methoxyl group (OCH_3) at δ_{H} 3.88 (s) and one proton of the CHO group at δ_{H} 9.82 (s) on a benzofuran moiety. Another pair of

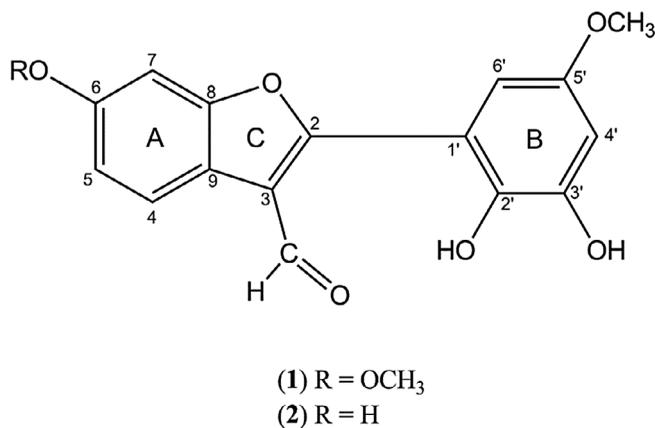


Figure 1. The structures of the isolated compounds.

meta-coupled aromatic protons were evident at δ_{H} 6.34 (d, $J = 2.2$ Hz, H-4') and 6.68 (d, $J = 2.2$ Hz, H-6') along with a OCH_3 group at δ_{H} 3.83 (s), which indicated a 1,2,3,5-tetrasubstituted benzene in the B-ring. The ^{13}C NMR data showed peaks characteristic of arylbenzofuran moiety following the signals observed at δ_{C} 133.8 (C-4), 109 (C-5), 100.6 (C-7), 98.9 (C-4') and 157.4 (C-6'). Thus, based on the ^1H and ^{13}C NMR data (Table S1), Compound **1** was strongly suggested to be a 2-arylbenzofuran.

Further characterisation of Compound **1** was performed using heteronuclear multiple bond correlation (HMBC) analysis (Figure S1). The placement of the OCH_3 and OH groups in the A- and B-rings was determined by HMBC. The long-range correlations between H- OCH_3 (A-ring, δ_{H} 3.88) and C-6 (δ_{C} 160), H- OCH_3 (B-ring, δ_{H} 3.83) and C-5' (δ_{C} 161.9) and H-OH (B-ring, δ_{H} 10.21) and C-1' (δ_{C} 107.8)/C-4' (δ_{C} 98.9) supported the assigned positions for the OCH_3 and OH groups in the A and B-rings, respectively. The assignment of the CHO group at C(3) also was determined by HMBC, which displayed a cross-peak between the CHO group at C-3 (δ_{C} 119.2) and C-1' (δ_{C} 107.8). This conclusion was supported by nuclear Overhauser enhancement (NOE) analysis (Figure S2). In this spectrum, a strong NOE association of H-C(4)/H-CHO(3); H-C(7)/MeO-C(6); H-C(4')/MeO-C(5') and H-C(6')/MeO-C(5'), established the location of the CHO and the OCH_3 groups, respectively.

From the above spectroscopic analyses, sesbagrandiflorain A was identified as 6-methoxy-2-(2',3'-dihydroxy-5'-methoxyphenyl)-1-benzofuran-3-carbaldehyde (Compound **1**).

Sesbagrandiflorain B (Compound **2**) was also isolated as a needle-like yellow crystal, and its molecular formula was determined as $\text{C}_{16}\text{H}_{12}\text{O}_6$ from ESI-TOF-MS, which exhibited $[\text{M} - \text{H}]^+$ at m/z 299.0554. The ^1H NMR spectral data of Compounds **1** and **2** displayed the same substitution-pattern signals of the 2-arylbenzofuran skeleton except for the hydroxyl group at C-6 on the A-ring. Similar features shown by Compounds **1** and **2** was also supported by comparison of their ^{13}C NMR spectral data (Table S1). The assignment of the OH group was afforded from the results of HMBC analysis (correlations: HO-C(6)/C(6); H-C(5)/C(6); and H-C(4)/C(6)) and nuclear Overhauser enhancement (NOE) spectroscopy data (NOE associations: H-C(7)/HO-C(6)). The HMBC correlation and the NOE associations of Compound **2** are shown in Figure S3 and S4, respectively.

Compound **2** displayed very similar one- and two-dimensional NMR profiles to those of Compound **1**. The only difference was that Compound **1** had a OCH_3 group attached at C-6 (A-ring) rather than the OH group found in Compound **2** at the same position. Therefore, Compound **2** was characterised as 6-hydroxy-2-(2',3'-dihydroxy-5'-methoxyphenyl)-1-benzofuran-3-carbaldehyde (sesbagrandiflorain B). This type of 2-arylbenzofuran compound was isolated previously by Tanaka et al. (2004) from the roots of *Erythrina variegata*. This is the first report of the occurrence of 2-arylbenzofurans in the stem bark of *S. grandiflora*.

3. Experimental

3.1. General methods

Thin-layer chromatography (TLC) was conducted on pre-coated silica gel 60 GF₂₅₄ plates (Merck, Darmstadt, Germany) with an absorbent thickness of 0.25 mm sprayed with $\text{Ce}(\text{SO}_4)_2$ solution for spot visualisation. In addition, preparative TLC was performed on square glass plates with a side length of 0.2 m coated with 0.5 mm Kieselgel F₂₅₄ (Merck), which were

air-dried and used without prior activation. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh ASTM; Merck).

Nuclear magnetic resonance (NMR) spectra were recorded for specimens dissolved in acetone- d_6 , with tetramethylsilane as an internal standard, using 500 MHz (^1H) and 125 MHz (^{13}C) spectrometers (Agilent Technologies, Santa Clara, CA, USA). Electrospray ionisation time-of-flight mass spectrometry (ESI-TOF-MS) was performed using a microTOF-Q IITM mass spectrometer (Bruker Daltonics Inc., Billerica, MA, USA). Potassium bromide-type infrared (IR) spectra were recorded using a Nicolet Avatar 360 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Finally, ultraviolet (UV) spectra were recorded using a UV-Vis Cary 100 spectrophotometer (Agilent Technologies).

3.2. Plant material

Samples of the stem bark of *S. grandiflora* were collected on June 2015 in Labuhan Ratu, Bandar Lampung, Indonesia. The identity of the plant specimens (No. N-IV) was confirmed at the Herbarium Bogoriense, Research Centre for Biology, Indonesian Institute of Sciences, Bogor, Indonesia. A voucher specimen was deposited at the herbarium.

3.3. Extraction and isolation

Air-dried and powdered samples of the stem bark of *S. grandiflora* (1.5 kg) were extracted exhaustively with *n*-hexane, EtOAc and 90% aqueous methanol (MeOH) sequentially at room temperature. Extraction was repeated three times, and the extracts were filtered and evaporated using a rotary vacuum evaporator at 40°C. The masses of the *n*-hexane, EtOAc and MeOH extracts obtained were 15, 60 and 53 g, respectively. The EtOAc extract was selected for further analysis. The EtOAc extract was fractionated by silica-gel vacuum liquid chromatography and eluted with EtOAc:*n*-hexane with a volume ratio ranging from 0–100%, yielding nine major fractions (*Fr.*), *Fr. E1–E9*. *Fr. E7* (237 mg) was further fractionated by CC on silica gel, and eluted with an acetone:*n*-hexane (2:98–65:35 v/v) gradient to yield 87 sub-fractions (*Fr. E7.1–E7.87*). A yellow crystal (Compound **1**) was obtained from sub-fractions *Fr. E7.38–E7.52* after recrystallisation with acetone:*n*-hexane (3:7 v/v). The total amount of Compound **1** obtained was 5.2 mg. The fractionation of *Fr. E3* (325 mg) was performed in the same way, using the eluent of an acetone:*n*-hexane (5:95–4:60 v/v) gradient to yield 42 sub-fractions. The fractions were examined by TLC, and those with identical profiles were combined to give three major sub-fractions (*Fr. E3.1–E3.3*). *Fr. E3.2* (38 mg) was further purified by preparative TLC (plates: 20 × 7 × 0.5 mm) using an acetone:*n*-hexane (3:7 v/v) gradient to provide Compound **2** (4.9 mg).

3.3.1. *Sesbagrandiflorain A (1): 6-methoxy-2-(2',3'-dihydroxy-5'-methoxyphenyl)-1-benzofuran-3-carbaldehyde*

$\text{C}_{17}\text{H}_{14}\text{O}_6$, needle-like yellow crystal, IR (KBr) ν_{max} cm^{-1} 3407, 2924, 1652, 1438, m.p. 215–216°C, UV (methanol) λ_{max} 213, 264, 348, 363, ESI-TOF-MS m/z 313.0709 [$\text{M} - \text{H}$]⁺, calculated 313.0718, ^1H NMR (Acetone- d_6) 3.83 (1H, s, H-OCH₃), 3.88 (1H, s, H-OCH₃), 6.34 (1H, d, $J = 2.2$ Hz, H-4'), 6.67 (1H, dd, $J = 2.2, 8.4$ Hz, H-5), 6.68 (1H, d, $J = 2.2$ Hz, H-6'), 6.71 (1H, d, $J = 2.2$ Hz, H-7), 7.55 (1H, d, $J = 8.4$ Hz, H-4), 9.82 (1H, s, H-CHO), 10.21 (1H, s, H-OH), ^{13}C NMR (Acetone- d_6): 56.1 (C-OCH₃), 56.2 (C-OCH₃), 88.5 (C-6'), 98.9 (C-4'), 100.6 (C-7), 107.8 (C-1'), 109.0 (C-5), 109.3

(C-9), 119.2 (C-3), 133.8 (C-4), 152.8 (C-3'), 157.4 (C-2'), 160.0 (C-6), 162.0 (C-5'), 162.9 (C-8), 164.4 (C-2), 191.1 (CHO) (Table S1).

3.3.2. *Sesbagrandiflorain B (2): 6-hydroxy-2-(2',3'-dihydroxy-5'-methoxyphenyl)-1-benzofuran-3-carbaldehyde*

$C_{16}H_{12}O_6$, needle-like yellow crystal, IR (KBr) ν_{\max} cm^{-1} 3461, 1640, m.p. 172–173°C, UV (methanol) λ_{\max} 215, 265, 348, 368; ESI-TOF-MS m/z 299.0554 [M – H]⁺, calculated 299.0561, ¹H NMR (Acetone- d_6) 3.83 (1H, s, H–OCH₃), 6.34 (1H, d, J = 2.2 Hz, H-4'), 6.61 (1H, dd, J = 2.2, 8.4 Hz, H-5), 6.64 (1H, d, J = 2.2 Hz, H-7), 6.67 (1H, d, J = 2.2 Hz, H-6'), 7.53 (1H, d, J = 8.4 Hz, H-4), 9.23 (1H, s, H-OH), 9.42 (1H, s, H-OH), 9.97 (1H, s, H-CHO), 10.26 (1H, s, H-OH), ¹³C NMR (Acetone- d_6): 56.1 (C–OCH₃), 88.5 (C-6'), 98.8 (C-4'), 104.2 (C-7), 107.9 (C-1'), 108.3 (C-9), 109.2 (C-5), 118.7 (C-3), 133.5 (C-4), 152.8 (C-3'), 157.3 (C-2'), 157.9 (C-6), 161.9 (C-5'), 162.5 (C-8), 164.7 (C-2), 191.3 (CHO) (Table S1).

4. Conclusions

In this study, two new 2-arylbenzofuran compounds, namely sesbagrandiflorain A and B, were obtained from the EtOAc extract of *S. grandiflora* stem bark. These compounds are reportedly present in the genus *Sesbania* and other members of the family Fabaceae. The bioactivity of both isolated compounds remains under investigation. Our findings expand our understanding of the natural constituents of the Fabaceae and, in particular, the *Papilionoideae* genera.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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