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A new lignan from the flowers of *Hibiscus sabdariffa* L. (Malvaceae)

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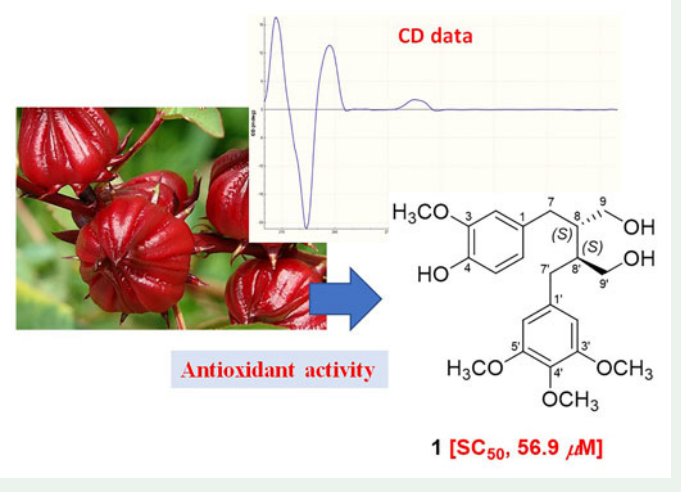
ABSTRACT

From MeOH-soluble fraction of the flowers of *Hibiscus sabdariffa* (Malvaceae), one new lignan, (+)-4-*O*-methyl-5′-methoxy-secoisolariciresinol (**1**), together with four known compounds (**2**–**5**) were isolated. The structures were elucidated based on NMR spectroscopic analysis. The absolute configuration of **1** was determined based on the Cotton effects in the CD spectrum. Compounds **1**, **3** and **4** showed antioxidant activities with the SC_{50} values of 56.9, 19.3 and 22.7 μM, respectively.



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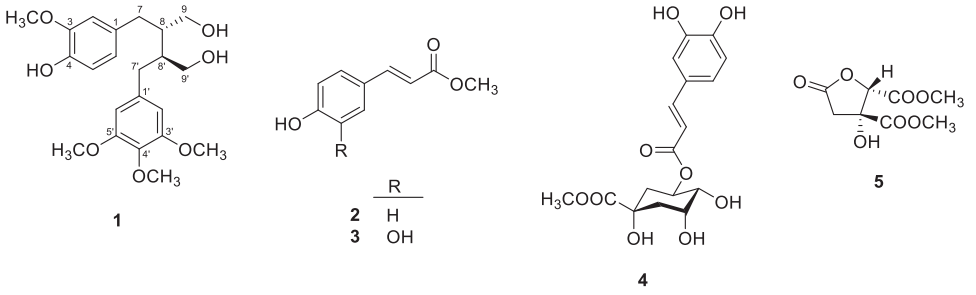

Figure 1. Structure of compounds **1**–**5**.

1. Introduction

Hibiscus sabdariffa L. (Malvaceae), also known as roselle, is an annual or perennial crop for developing countries as it is relatively easy to grow. It can be used as food, drinking infusion, and bast fibre (Da-Costa-Rocha et al. 2014). In Vietnam, *H. sabdariffa* is a local soft drink and medicinal herb for the treatment of hypertension, dyslipidemia, diabetes, fever, cardiovascular, and liver (Pham 2003). The main constituents of *H. sabdariffa* are organic acids, anthocyanins, polysaccharides and flavonoids (Ali et al. 2005; Riaz and Chopra 2018).

Roselle flower has gained attention for its antioxidant activity, which is predominantly contributed by anthocyanins (Maciel et al. 2018). The methanolic extract of roselle flowers showed the DPPH radical-scavenging activity with an SC_{50} value of 107.93 μg mL^{-1}. Thus, the further phytochemical investigation on this extract was carried out, that resulted in the isolation of a new lignan, (+)-4-*O*-methyl-5′-methoxy-secoisolariciresinol (**1**), along with four known compounds (**2**–**5**). Details regarding the isolation, structural elucidation and antioxidant activity of **1**–**5** are reported herein.

2. Results and discussion

The dried flowers of *H. sabdariffa* were extracted in turn with *n*-hexane, EtOAc and MeOH, to yield *n*-hexane-(SC_{50},>2000 μg mL^{-1}), EtOAc-(SC_{50}, 213.1 μg mL^{-1}), MeOH-soluble (SC_{50}, 107.93 μg mL^{-1}) fractions. Radical-scavenging activity-guided fractionation of MeOH-soluble fraction led to the isolation of five compounds: (+)-4-*O*-methyl-5′-methoxy-secoisolariciresinol (**1**), methyl *p*-coumarate (**2**) (Hosseini et al. 2016), methyl caffeate (**3**) (Lima et al. 2018), 5-*O*-caffeoylquinic acid methyl ester (**4**) (Chen et al. 2014) and hibiscus acid dimethyl ester (**5**) (Ibnusaud et al. 2002) (Figure 1).

Compound **1**, (+)-4-*O*-methyl-5′-methoxy-secoisolariciresinol, showed a *quasi*-molecular ion peak at *m/z* 407.2086 $[M+H]^+$ (calcd for $C_{22}H_{31}O_7$, 407.2070) in the HRESIMS. The IR spectrum exhibited absorptions of hydroxy groups (3354 cm^{-1}) and phenyl rings (1523 and 1455 cm^{-1}). The ^1H NMR spectrum showed signals for two sets of aromatic moieties, a 1,3,4-trisubstituted [δ_H 6.81 (d, $J = 8.0$ Hz, H-5), 6.63 (dd, $J = 8.0$, 1.9 Hz, H-6), 6.60 (d, $J = 1.9$ Hz, H-2)] and a 1,3,4,5-tetrasubstituted [δ_H 6.33 (2H, s, H-2′ and H-6′)], two oxymethylene groups [δ_H 3.85 (m, H-9′a), 3.56 (dd, $J = 4.6$, 1.9 Hz, H-9′b), 3.83 (m, H-9a), 3.58 (dd, $J = 4.6$, 1.9 Hz, H-9b)], two methine protons [δ_H 1.89 (2H, m, H-8 and H-8′)], two methylene groups [δ_H 2.76 (dd, $J = 13.8$, 6.7 Hz, H-7′a), 2.66

Table 1. DPPH radical-scavenging activity of the isolated compounds **1**–**5**.

Compound	SC_{50} (μM)	Compound	SC_{50} (μM)
1	56.9	**4**	22.7
2	–	**5**	–
3	19.3	Trolox[a]	38.0

[a]Positive control.

(dd, $J = 13.8$, 2.8 Hz, H-7′b), 2.75 (dd, $J = 13.8$, 6.6 Hz, H-7a), 2.67 (dd, $J = 13.8$, 2.6 Hz, H-7b)], as well as four methoxy groups [δ_H 3.82 and 3.80 (each 6 H, s)]. The ^{13}C NMR spectrum showed the presence of resonances for 12 aromatic carbons (δ_C 106.2–153.3), two oxymethylene carbons (δ_C 61.2, 61.1), two methylene carbons (δ_C 36.8, 36.1), two methine carbons (δ_C 44.1, 43.8), and four methoxy groups (δ_C 61.0, 2 × 56.3, 56.0) (supplementary Table S1). These were characteristic of those reported for secoisolariciresinol-type lignans (Agrawal and Thakur 1985; Sugahara et al. 2007), which were confirmed through analysis of the HMBC data. The HMBC correlations from the methoxy groups at δ_H 3.80 (6 H, s) to C-3′/C-5′ (δ_C 153.3), and at δ_H 3.82 (6 H, s) to C-3 (δ_C 146.7) and C-4′ (δ_C 136.5) were observed, which led to the assignment of the methoxy groups at C-3′/C-5′, C-4′ and C-3 (supplementary Figure S1). The CD spectrum of **1** showed the positive Cotton effects at 207, 236 and 284 nm, and a negative Cotton effect at 224 nm (supplementary Figure S2) revealed that the absolute configuration of **1** was identical to that of (+)-seco-5′-methoxy-isolariciresinol-9′-*O*-L-rhamnopyranoside as the 8*S* and 8′*S* form (Baek et al. 2018). It was also supported by the positive specific rotation value of $[\alpha]_D^{25}$ +33.7 (*c* 0.1, MeOH) (Baek et al. 2018). Accordingly, the structure of **1** was determined to be (+)-4-*O*-methyl-5′-methoxy-secoisolariciresinol.

All isolated compounds were tested for their DPPH radical-scavenging activity (Table 1). Trolox was a water-soluble derivative of vitamin E with potent antioxidant properties, which was served as positive controls with an SC_{50} value of 38.0 μM. The new lignan **1** exhibited weak activity with an SC_{50} value of 56.9 μM. Additionally, compounds **3** and **4** showed potent effects with the SC_{50} values of 19.3 and 22.7 μM, respectively.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on an A. Krüss Optronic polarimeter P3000 (Krüss Optronic GmbH). Circular dichroism spectrum was recorded on a Chirascan qCD spectrometer (Applied Photophysics Ltd.). Optical density values were determined with a 96-well micro-titer plate reader (Synergy HT, Biotek Instruments). The IR spectra were measured with a Shimadzu IR-408 infrared spectrometer (Shimadzu Pte., Ltd.). The NMR spectra were recorded on a Bruker Avance III 500 spectrometer (Bruker BioSpin AG) with deuterated solvents as the internal standard, and the chemical shifts are expressed as δ values (ppm). HRESIMS data were acquired on Bruker micrOTOF QII (Bruker Singapore Pte., Ltd.) mass spectrometer. Column chromatography was carried out using silica gel 60, 0.06–0.2 mm (Scharlab, S.L.), LiChroprep® RP-18, 40 − 63 μm (Merck KGaA), and Sephadex LH-20 (GE Healthcare Life Sciences). Analytical and

preparative TLC were carried out on precoated Kieselgel 60 F_{254} or RP_{18} plates (Merck KGaA).

3.2. Plant material

The flowers of *H. sabdariffa* L. were collected at Tuy Phong District, Binh Thuan Province, Vietnam, in December 2015 to January 2016. The sample was identified by MSc. Viet Hoang, Department of Ecology and Evolutionary Biology, Faculty of Biology and Biotechnology, VNUHCM–University of Science. A voucher specimen (DOC2016-HBG) has been deposited at the Department of Organic Chemistry, Faculty of Chemistry, VNUHCM-University of Science.

3.3. Extraction and isolation

The dried flowers of *H. sabdariffa* (3.5 kg) were refluxed in turn with *n*-hexane, EtOAc, and MeOH (each 30 L, 4 h × 3), to obtain the *n*-hexane- (124.8 g; SC_{50}, >2000 μg mL^{-1}), EtOAc- (67.2 g; SC_{50}, 213.10 μg mL^{-1}), and MeOH-soluble (409.1 g; SC_{50}, 107.93 μg mL^{-1}) fractions. The MeOH-soluble fraction was subjected to a silica gel column and eluted with MeOH–EtOAc (v/v, 0:100 → 0:100) mixtures, to obtain 11 fractions (Fr.1, 1976.33 μg mL^{-1}; Fr.2, 165.07 μg mL^{-1}; Fr.3, 204.27 μg mL^{-1}; Fr.4, 145.00 μg mL^{-1}; Fr.5, 154.70 μg mL^{-1}; Fr.6, 169.47 μg mL^{-1}; Fr.7, 177.60 μg mL^{-1}; Fr.8, 245.80 μg mL^{-1}; Fr.9, 181.83 μg mL^{-1}; Fr.10, 233.17 μg mL^{-1}; Fr.11, 193.73 μg mL^{-1}). Fraction Fr.4 (2.1 g) was separated over a silica gel column with MeOH–EtOAc (v/v, 0:100 → 0:100) mixtures, to give 5 subfractions (Fr.4.1, 181.77 μg mL^{-1}; Fr.4.2, 287.33 μg mL^{-1}; Fr.4.3, 18.96 μg mL^{-1}; Fr.4.4, 60.88 μg mL^{-1}; Fr.4.5, 15.86 μg mL^{-1}). Subfraction Fr.4.3 (540 mg) was chromatographed over a silica gel column with MeOH-$CHCl_3$ (v/v, 0:100 → 100:0) mixtures and purified by preparative TLC with Me_2CO-$CHCl_3$ (3:7), to afford **1** (3.5 mg), **2** (5.0 mg), **3** (3.0 mg), **4** (3.0 mg) and **5** (3.5 mg).

3.3.1. (+)-4-O-Methyl-5′-methoxy-secoisolariciresinol (1)

Colorless gum; $[\alpha]_D^{25}$ +33.7 (*c* 0.1, MeOH); CD (*c* 0.1, MeOH) λ_{max} nm (θ, mdeg) 207 (+19.3), 224 (−25.1), 236 (+13.5), 284 (+2.1); IR (KBr) ν_{max} 3354, 1523, 1455 cm^{-1}; ^1H (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see supplementary Table S1; HRESIMS *m/z* 407.2086 $[M+H]^+$ (calcd for $C_{22}H_{31}O_7$, 407.2070).

3.4. DPPH radical-scavenging activity assay

The DPPH radical-scavenging capacity assay was performed as the previous method (Herald et al. 2012) with some modifications. A solution of DPPH 0.15 mM in 80% methanol was prepared daily. A 25 μL of a sample at various concentrations was allowed to react with 200 μL of DPPH solution in the dark for 1 h at ambient temperature. Then, the solution absorbance at 517 nm was measured. All solutions were measured against an 80% methanol blank. The % DPPH quenched was determined according to the equation: % Scavenging activity = [1 − (OD_{sample} − OD_{blank})/($OD_{control}$ − OD_{blank})] × 100. The SC_{50} value (μM) was defined as the concentration of

test sample required to scavenge 50% of DPPH free radicals. This value was determined by the multiple nonlinear regression method (GraphPad Prism software). Trolox was used as a positive control at 5–80 μmol L^{-1} to generate a calibration curve ($R^2 > 0.9$).

4. Conclusions

The radical-scavenging activity-guided isolation of the flowers of *H. sabdariffa* was carried out to obtain five compounds (**1**–**5**). The absolute configuration of the new lignan, (+)-4-*O*-methyl-5′-methoxy-secoisolariciresinol (**1**), was identified based on NMR interpretation and CD Cotton effects. Compounds **1**, **3** and **4** showed promising antioxidant activity with the SC_{50} values of 56.9, 19.3 and 22.7 μM, respectively.

Disclosure statement

No potential conflict of interest was reported by the authors.

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