Two new derivatives of 8-prenyl-5,7-dihydroxycoumarin from the stems of Streblus ilicifolius (S.Vidal) Corn

Nhan Trung Nguyen\textsuperscript{a,b,c}, Hai Xuan Nguyen\textsuperscript{a,b}, Tho Huu Le\textsuperscript{a,b}, Du Huy Nguyen\textsuperscript{a,b}, Truong Nhat Van Do\textsuperscript{a,b}, Phu Hoang Dang\textsuperscript{a,b} and Mai Thanh Thi Nguyen\textsuperscript{a,b,c}

\textsuperscript{a}Faculty of Chemistry, University of Science, Ho Chi Minh City, Vietnam; \textsuperscript{b}Vietnam National University, Ho Chi Minh City, Vietnam; \textsuperscript{c}Cancer Research Laboratory, University of Science, Ho Chi Minh City, Vietnam

\textbf{ABSTRACT}

From the EtOAc-soluble extract of the stems of \textit{Streblus ilicifolius} (Moraceae), two new secondary metabolites named strebluses A (1) and B (2) were isolated. Their chemical structures have been concluded based on the chemical derivatisation and the spectroscopic interpretation. All compounds have been tested for their tyrosinase inhibitory activity. They showed weaker inhibitory activity than that of kojic acid (IC\textsubscript{50}, 44.6 \textmu M).

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\section{1. Introduction}

\textit{Streblus ilicifolius} (S.Vidal) Corn. (Moraceae), known as a scandent spinous shrub, growing up to 2.5–3 m high, is widely distributed in India, China, and South Asia (Singh et al. 2012). In Vietnam, this plant which is locationally called the trivial name ‘Duoi O-ro’, was planted as a fence and its bark is a folk medicine for treating pimples (Do 2006). Several previous phytochemical studies on \textit{S. ilicifolius} stems revealed that it had contained some of coumarins, stilbenes, lignans, and polyphenols and its
biological activities on anti-tyrosinase and antimicrobial activities had been reported (Dej-adisai et al. 2016; Zhang et al. 2019).

By a continuing effort on the inhibition of melanin synthesis of the indigenous medicinal plants of Vietnam (Nguyen et al. 2016; Nguyen et al. 2012), the MeOH-soluble extract of the stems of *S. ilicifolius* showed significant tyrosinase inhibitory activity with an IC$_{50}$ value of 6.3 µg mL$^{-1}$. Further biological study on the EtOAc-soluble fraction exhibited more potent inhibitory effect with an IC$_{50}$ value of 4.9 µg mL$^{-1}$. Thus, its phytochemical investigation was carried out, leading to the isolation of two new compounds, strebluses A (1) and B (2) (Figure 1). In this study, we have reported the isolation and the structure elucidation of both new compounds along with their tyrosinase inhibitory activity.

2. Results and discussion

Compound 1 was obtained as a white amorphous powder. The HRESIMS data gave a sodium adduct molecular ion peak at *m/z* 287.0900 [M+Na]$^+$ (calcd. for 287.0895, C$_{14}$H$_{16}$O$_{5}$Na). The IR spectrum indicated the presence of hydroxy (3398 cm$^{-1}$), carbonyl (1693 cm$^{-1}$), double bond (1612 cm$^{-1}$), and phenyl (1566 cm$^{-1}$) groups. The $^1$H NMR spectrum of 1 displayed signals of an isolated aromatic proton at $\delta_H$ 6.44 (1H, s, H-6), two cis-coupled olefinic protons at $\delta_H$ 6.04 (1H, d, $J=9.6$ Hz, H-3) and 8.04 (1H, d, $J=9.6$ Hz, H-4), together with one oxymethylene ($\delta_H$ 3.49 and 3.36, H$_2$-4'), one methine ($\delta_H$ 1.63, H-3'), two methylenes ($\delta_H$ 2.74, H$_2$-1'; 1.68 and 1.36, H$_2$-2'), and one methyl ($\delta_H$ 0.99, H$_3$-5') (Supplementary data Table S1). On the other hand, the $^{13}$C NMR spectrum of 1 showed resonance signals for 14 carbons including an ester carbonyl carbon ($\delta_C$ 160.7, C-2), three oxygenated aromatic carbons ($\delta_C$ 153.2, C-5; 159.4, C-7; 154.5, C-9), two tertiary aromatic carbons ($\delta_C$ 108.1, C-8 and 102.3, C-10), an methine aromatic carbon ($\delta_C$ 98.0, C-6), along with one oxymethylene ($\delta_H$ 67.4, C-4'), one methine ($\delta_H$ 10.7, C-1') and 33.2, C-2'), and one methyl ($\delta_H$ 16.4, C-
5) (Supplementary data Table S1). These $^1$H and $^{13}$C NMR data together with the observed HMBC correlations indicated 1 having the coumarin moiety. The location of two hydroxy groups was determined to be at C-5 and C-7 based on the HMBC correlations from H-6 to both C-5 and C-7. The presence of the 4-hydroxy-3-methylbutyl group was suggested on the basis of the HMBC correlations from the oxymethylene protons H$_2$-4' to the methylene carbon C-2', the methine carbon C-3', and the methyl carbon C-5'. In addition, its position was determined at C-8 by the HMBC correlations from the oxymethylene protons H$_2$-1' to the aromatic carbons C-7, C-8, and C-9, and from the oxymethylene protons H$_2$-2' to the aromatic carbon C-8. The methylation derivatisation of 1 was carried out to obtain a dimethylated product 1'. The structure of 1' was concluded based on the $^1$H and $^{13}$C NMR data (Supplementary data Table S1) and its HMBC correlations (Supplementary data Figure S1). The NOESY correlations of 1' were used to confirm the position of the 4-hydroxy-3-methylbutyl group in 1. Compound 1' showed the NOESY correlations between H-6/5-OMe, H-6/7-OMe, and H$_2$-1'/7-OMe, which identified the location of the 4-hydroxy-3-methylbutyl group at C-8. Thus, the structure of streblus A (1) was concluded as 5,7-dihydroxy-8-(4'-hydroxy-3'-methylbutyl)coumarin.

Compound 2 was isolated as a white amorphous powder. The molecular formula of 2 was determined to be C$_{16}$H$_{18}$O$_6$ by the HRESIMS. The $^1$H and $^{13}$C NMR spectra of 2 resembled closely those of 1, except for the presence of the acetyl group ($\delta_H$ 1.98; $\delta_C$ 171.2, 20.8) (Supplementary data Table S1). The HMBC correlation between the oxymethylene protons H$_2$-4' and the acetoxy carbonyl at $\delta_C$ 171.2 suggested that the location of acetylation is at C-4' (Supplementary data Figure S1). The methylation of 2 was carried out to afford a dimethylated product 2'. The NOESY spectra of 2' showed the correlations between H-6/5-OMe, H-6/7-OMe, and H$_2$-2'/7-OMe. Thus, the 4-acetoxy-3-methylbutyl group was determined to be at C-8. The structure of streblus B (2) was concluded as 5,7-dihydroxy-8-(4'-acetoxy-3'-methylbutyl)coumarin. The HPLC data of 1 and the MeOH-soluble extract (Supplementary data Figure S24) showed the presence of 1 and 2 in this extract. Thus, possibility of 2 to be an artifact can be ignored.

Two isolated compounds were tested for their tyrosinase inhibitory activity according to the modified method of Arung et al. (2005). Kojic acid (IC$_{50}$, 44.6 µM), which is a well-known tyrosinase inhibitor (Hae et al. 2019), was used as the positive control. The assay was carried out at various concentrations ranging from 10 to 100 µM. Compounds 1 and 2 at the concentration of 100 µM showed weak effect with the inhibitory percentage values of 26.9% and 21.1%, respectively.

3. Experimental
3.1. General experimental procedures

Optical rotation was recorded on a Jasco P-2000 digital polarimeter (JASCO International Co., Ltd., Japan). HRESIMS was performed on a Bruker microTOF-QII mass spectrometer (Bruker Singapore Pte., Ltd., Singapore). IR spectra was measured with a JASCO FT/IR-6600 spectrometer (JASCO International Co., Ltd., Japan) with KBr as a carrier for the sample. NMR spectra was taken on a Bruker Avance III 500 spectrometer (Brucker BioSpin AG, Thailand) with acetone-$d_6$ as an internal standard, and chemical
shifts are expressed in $\delta$ values. HPLC data were carried out on Agilent 1260 Infinity II LC System with diode array detector (Agilent Technologies, Pte Ltd., Singapore). The absorbance (OD) was measured with a Shimadzu UV-1800 UV-Vis spectrophotometer (Shimadzu Pte., Ltd, Singapore). Column chromatography was carried out using Silica gel 60, (40–63 $\mu$m) (Scharlau, Spain). Analytical and preparative TLCs were carried out on precoated Kieselgel 60 F254 plate (Merck KGaA, Germany). Mushroom tyrosinase (EC 1.14.18.1) (3933 U/mL) and L-3,4-dihydroxyphenylalanine (L-DOPA) were purchased from Sigma-Aldrich (Sigma-Aldrich Pte. Ltd., Singapore). Kojic acid and DMSO were purchased from Merck (Merck KGaA, Germany). Other chemicals were of the highest grade available.

3.2. Plant material

The stems of *S. ilicifolius* were collected at Hoai Nhon District, Binh Dinh Province, Vietnam in October 2017. It was identified by Dr. rer. nat. Anh Tuan Dang-Le, Faculty of Biology and Biotechnology, University of Science, Ho Chi Minh City, Vietnam. A voucher sample of the stems (MCE0052) has been deposited at the Department of Medicinal Chemistry, Faculty of Chemistry, University of Science, Ho Chi Minh City, Vietnam.

3.3. Extraction and isolation

A part of the dried powder stems of *S. ilicifolius* (100.0 g) were exhaustively extracted in a Soxhlet extractor with MeOH to yield the MeOH-soluble extract (10.2 g) for screening biological activity. The powdered materials of *S. ilicifolius* (10.0 kg) were done similarly with $n$-hexane, EtOAc, and MeOH to yield $n$-hexane- (64.8 g), EtOAc- (117.2 g), and MeOH (378.0 g)-soluble fractions, respectively. The EtOAc-soluble fraction was subjected to a silica gel column and eluted with MeOH–CHCl$_3$ mixtures (v/v, 0:100, 1:99, 3:97, 5:95, 10:90, 15:85, 20:80, 30:70, 40:60, 50:50, 70:30) to obtain 18 fractions: fr.1 (0.9 g), fr.2 (1.2 g), fr.3 (0.4 g), fr.4 (1.9 g), fr.5 (4.4 g), fr.6 (2.5 g), fr.7 (1.2 g), fr.8 (0.8 g), fr.9 (1.1 g), fr.10 (1.0 g), fr.11 (4.7 g), fr.12 (1.6 g), fr.13 (4.4 g), fr.14 (19.6 g), fr.15 (20.1 g), fr.16 (25.8 g), fr.17 (8.2 g), and fr.18 (7.3 g). Fraction fr.12 was chromatographed using MeOH–CHCl$_3$ mixtures (v/v, 0:100 → 70:30) as the eluent to afford seven subfractions (fr.12-1, 101 mg; fr.12-2, 212 mg; fr.12-3, 140 mg; fr.12-4, 62 mg; fr.12-5, 55 mg; fr.12-6, 218 mg; fr.12-7, 606 mg). Subfraction fr.12-2 was subjected to a silica gel column by elution with EtOAc–$n$-hexane mixtures (v/v, 0:100 → 80:20), and then followed with acetone–CHCl$_3$ mixtures (v/v, 0:100 → 50:50) to furnish 2 (20.9 mg). Subfraction fr.13 was passed over a silica gel column eluted with MeOH–CHCl$_3$ mixtures (v/v, 0:100 → 70:30) to give nine subfractions (fr.13-1, 41 mg; fr.13-2, 225 mg; fr.13-3, 374 mg; fr.13-4, 343 mg; fr.13-5, 189 mg; fr.13-6, 526 mg; fr.13-7, 608 mg; fr.13-8, 611 mg; fr.13-9, 1 g). Subfraction fr.13-4 was further separated by silica gel column chromatography with acetone–$n$-hexane mixtures (v/v, 0:100 → 80:20), and then purified by preparative TLC with a MeOH–CHCl$_3$ mixture (5:95) to obtain 1 (5.1 mg).
3.3.1. *Streblus A* (1)
White amorphous powder; $[\alpha]_{D}^{25} + 49.9$ (c 0.005, MeOH); IR (KBr) $\nu_{\text{max}}$ 3398, 2927, 1693, 1612, 1566 cm$^{-1}$; $^1$H (500 MHz, acetone-$d_6$) and $^{13}$C (500 MHz, acetone-$d_6$) NMR: see Supplementary data Table S1; HRESIMS: m/z 287.0900 [M + Na]$^+$ (calcd for C$_{14}$H$_{16}$O$_5$Na$^+$, 287.0895).

3.3.2. *Streblus B* (2)
White amorphous powder; $[\alpha]_{D}^{25} + 27.0$ (c 0.01, MeOH); IR (KBr) $\nu_{\text{max}}$ 3248, 2962, 1693, 1612, 1566, 1257 cm$^{-1}$; $^1$H (500 MHz, acetone-$d_6$) and $^{13}$C (500 MHz, acetone-$d_6$) NMR: see Supplementary data Table S1; HRESIMS: m/z 329.1027 [M + Na]$^+$ (calcd for C$_{16}$H$_{18}$O$_6$Na$^+$, 329.1001).

3.4. *Hplc data of the MeOH-Soluble extract from S. ilicifolius*

The concentrations of the MeOH-soluble extract and two new compounds (1 and 2) were approximately 10,000, 1000, and 1000 ppm, respectively. The detection wavelength was set at 330 nm. An Agilent Zorbax SB-C18 column (150 × 4.6 × 5 mm) was used with a flow rate of 1 mL/min. The injection volume was 20 μL and the column temperature was maintained at 30°C. The mixtures of water and ACN were used as the mobile phase with gradient elution (20 → 30% ACN, for 15 min).

3.5. *General procedure for O-methylation*

Dissolved ~5.0 mg of each compound in 3 mL acetone, then added 5.0 mg K$_2$CO$_3$. The mixture was subsequently treated with 30 μL CH$_3$I and stirred for 2 h at room temperature in Ar atmosphere. The reaction mixture was added 10 mL water and 3 mL saturated NaCl solution, and then extracted three times with 3 mL EtOAc for each time. Finally, flash column chromatography was applied and eluted with EtOAc–n-hexane mixture (v/v, 20:80) to isolate the product.

3.5.1. *5,7-Di-O-methylstreblus A* (1′)
White amorphous powder; $^1$H (500 MHz, acetone-$d_6$) and $^{13}$C (500 MHz, acetone-$d_6$) NMR: see Supplementary data Table S1.

3.5.2. *5,7-Di-O-methylstreblus B* (2′)
White amorphous powder; $^1$H (500 MHz, acetone-$d_6$) and $^{13}$C (500 MHz, acetone-$d_6$) NMR: see Supplementary data Table S1.

3.6. *Tyrosinase inhibitory activity assay*

All the samples were first dissolved in DMSO and used for the actual experiment at concentrations of 1–100 μg/mL (or μM for pure compounds). The tyrosinase inhibitory activity assay was performed as previously described by Arung et al. (2005). The assay mixtures consisting of 1450 μL of test solution in 0.1 M phosphate buffer (pH = 6.8) and 50 μL of enzyme solution (15 U/mL in 0.1 M phosphate buffer) was prepared
immediately before use. After preincubation at room temperature for 30 min, the reaction was initiated by the addition of 500 μL of substrate solution (1.5 mM L-DOPA in 0.1 M phosphate buffer). The assay mixture was incubated at room temperature for 7 min, and then measured the absorbance (Abs) at 475 nm. Kojic acid, a known tyrosinase inhibitor, was used as a positive control. Tyrosinase inhibitory activity was expressed as the inhibitory percentage, calculated as \[(1 – \frac{Abs_{\text{sample}}}{Abs_{\text{control}}}) \times 100\%\]. Data were represented as means ± standard error (n = 3).

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**ORCID**

Nhan Trung Nguyen [http://orcid.org/0000-0001-5142-4573](http://orcid.org/0000-0001-5142-4573)
Hai Xuan Nguyen [http://orcid.org/0000-0001-8447-8367](http://orcid.org/0000-0001-8447-8367)
Tho Huu Le [http://orcid.org/0000-0001-9623-2858](http://orcid.org/0000-0001-9623-2858)
Truong Nhat Van Do [http://orcid.org/0000-0001-8538-7539](http://orcid.org/0000-0001-8538-7539)
Phu Hoang Dang [http://orcid.org/0000-0002-4989-9315](http://orcid.org/0000-0002-4989-9315)
Mai Thanh Thi Nguyen [http://orcid.org/0000-0001-8006-4028](http://orcid.org/0000-0001-8006-4028)

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