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Tyrosinase inhibitors from the heartwood of Vietnamese *Artocarpus heterophyllus*

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ABSTRACT

*Ethyl acetate extract of the Vietnamese heartwoods of Artocarpus heterophyllus has shown a good inhibition of tyrosinase with the IC_{50} value of 0.78 μg/mL. Investigation on the chemical constituents of this extract has resulted in the isolation of four compounds, cudraflavon B (**1**), brosimon I (**2**), artocarpin (**3**) and morachalcon A (**4**). Their structures were determined by a combination of HR-MS and 2D NMR spectroscopy. In addition, all four compounds **1**-**4** have inhibitory activity against tyrosinase. Especially, compound **4** has the strongest activity (IC_{50} value of 0.18 μg/mL).*

Key words: *Artocarpus heterophyllus*, heartwood, tyrosinase.

INTRODUCTION

Artocarpus heterophyllus is well-known in Vietnam and other countries in Asia as a fruit plant [1]. The fruit is edible and has a good taste. The whole areal parts of this plant have been used as medicinal materials [2]. Previous studies reported the isolation of various secondary metabolites which showed interestingly biological activities such as inhibition of NO production in RAW cells [3], inhibition of tyrosinase [4], cytotoxicity [5], antidiabetic activity [6]. Its latex also has antifungic against post harvest pathogens [7]. Recently, we found the EtOAc extract of the heartwood of *Artocarpus heterophyllus* could inhibited tyrosinase significantly. Chemical investigation of its EtOAc extract led to the isolation of four metabolites. This paper describes the isolation, structural elucidation and inhibition of tyrosinase of four compounds from the heartwood of Vietnamsese *Artocarpus heterophyllus*.

MATERIALS AND METHODS

Materials

Heartwood of *Artocarpus heterophyllus* Linn was collected Dien Bien province in June 2012 and identified by MSc. Nguyen The Anh (Institute of Chemistry, VAST, Vietnam). Voucher specimens are deposited at faculty of Chemistry, Hanoi University of Education (NHH-2012).

Methods

General

TLC was performed on silica gel plates (Kieselgel 60 F_{254}, Merck). Preparative HPLC was performed on a Jasco PU-2087 instrument with a UV-2070 and RI-2031 detectors using a Waters 5 SL-II column (10.0 x 250 mm), flow rate of 1.0 mL/min. NMR spectra were recorded on Varian Brucker Avance 500 MHz, using $CDCl_3$ as solvent. Chemical shifts are referenced to internal TMS (0 ppm, ^1H) and $CDCl_3$ (77.0 ppm, ^{13}C), respectively. The positive

ion high-resolution ESI-MS were recorded on a Bruker Apex III Fourier transform ion cyclotron resonance (FTICR) mass spectrometer, equipped with a 7 Tesla superconducting magnet.

Extraction and isolation

Dried powders of heartwood of *Artocarpus heterophyllus* (2940 g) were extracted with methanol (10L x 3). The methanolic extract was concentrated to give a residue (34.8g) which was further partitioned into *n*-hexane, EtOAc, BuOH and water. The EtOAc crude extract (5 g) was chromatographied by silica gel column, eluting by n-hexane/EtOAc gradient, followed by prep. HPLC with hexane/EtOAc (2/1) to afford four compounds, compound **1** (2.0 mg), compound **2** (2.0 mg), compound **3** (15.0 mg) and compound **4** (5 mg).

Compound **1**: ESI-FTICR-MS: m/z $[M-H]^-$ calcd for $C_{25}H_{23}O_6$: 419.1495; found 419.1493. ^{1}H NMR (500 MHz, $CDCl_3$): δ 13.16 (1H, s, 5-OH), 7.20 (1H, d, H-6'), 6.72 (1H, d, J= 10 Hz, H-1"'), 6.52 (1H, d, J = 2.5 Hz, H-3'), 6.50 (1H, dd, J = 8.5 and 2.5 Hz, H5'), 6.26 (1H, s, H-8), 5.60 (1H, d, J = 10 Hz, H-2"'), 5.16 (1H, m, H-2"), 3.12 (2H, d, J = 6.5 Hz, H-1"), 1.65 (3H, s, H-5"), 1.54 (3H, s, H-4"), 1.46 (6H, s, H-4''' and H-5''').

Compound **2**: ESI-FTICR-MS: m/z $[M-H]^-$ calcd for $C_{25}H_{23}O_6$: 419.1495; found 419.1498. ^{1}H NMR (500 MHz, $CDCl_3$): δ 13.25 (1H, s, 5-OH), 7.66 (1H, d, J = 7.0 Hz, H-6'), 6.55 (1H, dd, J = 8.5, 2.5 Hz, H-5'), 6.49 (1H, s, H-8), 6.41 (1H, d, J = 2.5 Hz, H-3'), 6.38 (1H, d, J = 16.5 Hz, H-1'''), 6.18 (1H, dd, J = 16.5, 6.5 Hz, H-2'''), 6.17 (1H, d, J = 10Hz, H-1"), 5.40 (1H, dd, J = 9.1, 1.0 Hz, H-2"), 2.56 (1H, m, H-3'''), 1.97 (3H, d, J = 1.0 Hz, H-5"), 1.70 (3H, d, J = 1.0 Hz, H-4"), 1.14 (6H, d, J = 6.5 Hz, H-4''' and H-5''').

Compound **3**: ESI-FTICR-MS: m/z $[M+H]^+$ calcd for $C_{26}H_{29}O_6$: 437.1964; found 437.1960. ^{1}H NMR (500 MHz, $CDCl_3$): δ 13.48 (1H, s, 5-OH), 7.19 (1H, d, J = 9.0 Hz, H-6'), 6.68 (1H, dd, J = 16.5, 7.0 Hz, H-2'''), 6.52 (1H, d, J = 16.5 Hz, H-1'''), 6.51 (1H, d, J = 2.5 Hz, H-3'), 6.50 (1H, dd, J = 9.9, 2.5 Hz, H-5'), 6.38 (1H, s, H-8), 5.14 (1H, m, H-2''), 3.87 (3H, s, 7-OMe), 3.13 (2H, d, J = 6.5 Hz, H-1''), 2.47 (1H, m, H-3'''), 1.63 (3H, s, H-5''), 1.45 (3H, s, H-4''), 1.10 (6H, d, J = 7.0 Hz, H-4''' and H-5'''). ^{13}C NMR: δ 182.3 (C-4), 163.0 (C-7), 159.5 (C-2), 159.1 (C-4'), 158.6 (C-5), 156.2 (C-8a), 155.2 (C-2'), 142.7 (C-2'''), 133.3 (C-3"), 131.6 (C-6'), 121.6 (C-3), 120.9 (C-2"), 115.6 (C-1'''), 112.6 (C-1'), 109.8 (C-6), 108.4 (C-5'), 105.0 (C-4a), 103.9 (C-3'), 89.5 (C-8), 56.0 (7-OMe), 33.1 (C-3'''), 25.7 (C-5"), 24.4 (C-1"), 22.7 (C-4''' and C-5'''), 17.7 (C-4").

Compound **4**: ESI-FTICR-MS: m/z 339.1248 ($[M-H]^-$) calcd for $C_{20}H_{19}O_5$: 339.1232. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.08 (1H, d, J = 15.5 Hz, Hβ), 7.65 (1H, d, J=9.0 Hz, H-6'), 7.42 (1H, d, J = 8.5 Hz, H-6), 7.67 (1H, d, J =15.5 Hz, Hα), 6.41 (1H, d, J= 8.5 Hz, H-5'), 6.40 (1H, dd, J = 8.5, 2.5 Hz, H-5), 6.34 (1H, m, H-3), 5.28 (1H, m, H-2"), 3.36 (2H, d, J= 10.5 Hz, H-1"), 1.81 (3H, s, H-5"), 1.69 (3H, s, H-4"). ^{13}C NMR: δ 193.3 (C=O), 164.5 (C-2'), 162.0 (C-4'), 160.0 (C-4), 159.0 (C-2), 140.8 (Cβ), 132.3 (C-3"), 131.8 (C-6), 129.2 (C-6'), 122.4 (C-2"), 117.9 (Cα), 115.5 (C-3'), 115.0 (C-1), 114.0 (C-1'), 108.3 (C-5), 107.2 (C-5'), 103.2 (C-3), 25.8 (C4"), 21.8 (C-1"), 17.9 (C-5").

Tyrosinase assay

The inhibition activity of tyrosinase of the crude extract and isolated compounds from the heartwood of *Artocarpus heterophyllus* was evaluated following the method written by Arung et al. [8].

RESULTS AND DISCUSSION

The ethyl acetate extract of the heartwood of *Artocarpus heterophyllus* was subjected to silica gel column chromatography and followed by prep. HPLC to give four aromatic compounds (**1**-**4**).

Compound **1** was obtained as white crystals. Its molecular formula was found to be $C_{25}H_{24}O_6$ by FT-ICR-MS. The ^{1}H NMR spectrum indicates the presence of flavonoid skeleton. This NMR spectrum also suggests that it possesses a prenyl group which is proven by the signals for two methyls at 1.46 and 1.54 ppm, one methylene at 3.12 ppm and one olefinic proton at 5.16 ppm. In addition, one hydroxyl group resonances at 13.16 ppm reveals that it is a hydrogen bonded hydroxyl group with the carbonyl group. Beside that, it NMR spectrum has signals for aromatic protons at 7.20 (1H, d, H-6'), 6.52 (1H, d, J = 2.5 Hz, H-3'), 6.50 (1H, dd, J = 8.5 and 2.5 Hz, H5') and 6.26 (1H, s, H-8). Furthermore, compound **1** has identical NMR spectral data with those of cudraflavon B [9]. Therefore, compound **1** is found to be cudraflavon B as shown in Fig. 1.

Compound **2** was also obtained from EtOAc extract of *Artocarpus heterophyllus*. Its ^{1}H NMR spectrum has signals of one hydrogen bonded hydroxyl group at 13.25 ppm, four aromatic protons at 7.66 (1H, d, J = 7.0 Hz, H-6'), 6.55 (1H, dd, J = 8.5, 2.5 Hz, H-5'), 6.41 (1H, d, J = 2.5 Hz, H-3') and 6.49 (1H, s, H-8); two vinylic methyls at 1.97 (3H, d, J = 1.0 Hz, H-5"), 1.70 (3H, d, J = 1.0 Hz, H-4"); and two secondary methyls at 1.14 (6H, d, J = 6.5 Hz, H-

4''' and H-5'''). In comparison its spectral data with those of brosimon I [10], we conclude that compound **2** is brosimon I.

The molecular formula of compound **3** was found to be $C_{26}H_{29}O_6$ by FT-ICR-MS. Analysis of its ^{1}H NMR spectra revealed that it also has a hydrogen bonded hydroxyl group at 13.48 (1H, s, 5-OH), one methoxyl group at 3.87 ppm and two prenyl groups. The ^{13}C NMR spectrum has resonances of 26 carbons, including one ketone group at 182.3 ppm, six phenolic carbons at 163.0, 159.5, 159.1, 158.6, 156.2, 155.2. Then, the structure of compound **3** is determined by 2D NMR such as HSQC and HMBC. Compound **3** has very similar spectral data with those of artocarpin [9]. Therefore, compound **3** was determined as artocarpin.

From the EI-MS data of **4** was afforded m/z 339.1248 ([M-H]$^-$), corresponding to a molecular formula of $C_{20}H_{19}O_5$. Therefore, compound **4** has molecular formula of $C_{20}H_{20}O_5$. The ^{1}H NMR spectrum indicates the presence of two *trans*-olefinic proton at 8.08 and 7.67 ppm (J = 15.5 Hz), one prenyl group and four aromatic protons. Analysis of ^{13}C NMR reveals that it possesses 20 carbon atoms. These NMR spectral data suggest that compound **4** is a chalcone. The prenyl group is located at C-3' due to the HMBC correlations between H-1" and H-2', H-4'. The ketone group is coupled to H-α, H-β, H-6'in its HMBC spectrum. From the mass, ^{1}H and ^{13}C NMR spectral data identified **4** as morachalcon A [11].

Previous studies showed that phenolic compounds are promising agents for whitening skin [13,14]. Then, the inhibition activity of tyrosinase of these isolated compounds (**1**-**4**) from heartwood of *Artocarpus heterophyllus* was evaluated. The obtained result is described in table 1. Accordingly, all four compound have good activity. Especially, morachalcon A (**4**) has strongest activity with the IC_{50} of 0.18 µg/mL, even stronger than acid kojic as a standard (IC_{50} = 3.97 µg/mL).

Figure 1. Structures of compounds 1-4

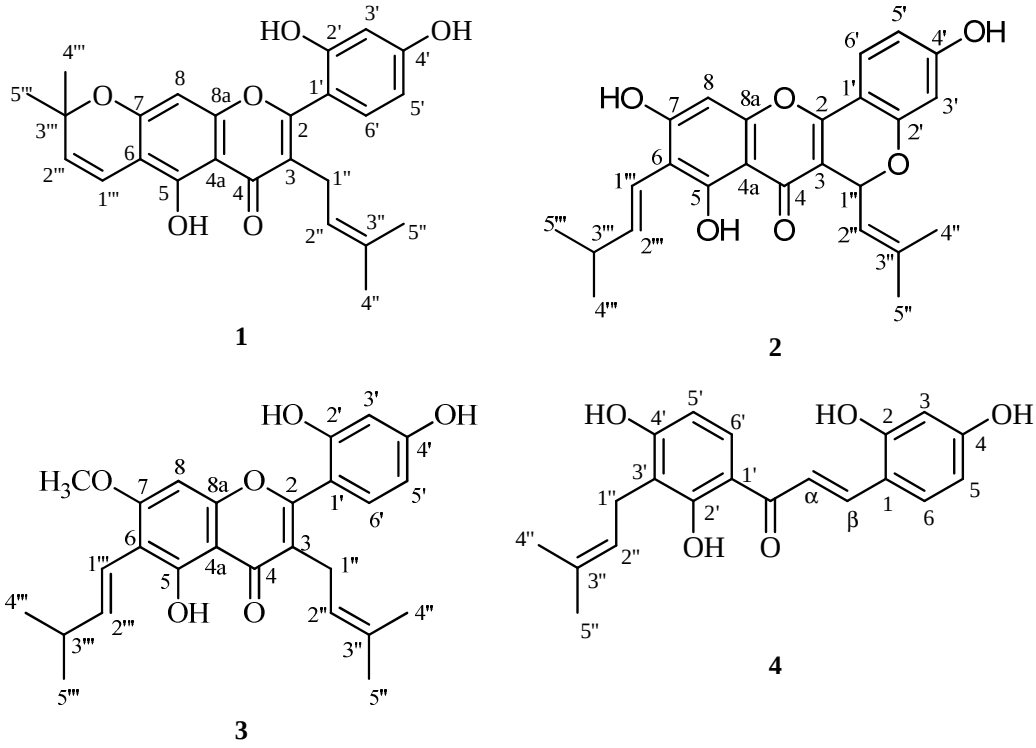

Table 1: Inhibition activity of tyrosinase of compounds (1-4) from *A. heterophyllus*

Samples/ Standard	IC_{50} (µg/mL)
Cudraflavon B (**1**)	1.03 ± 0.65
Brosimon I (**2**)	1. 78 ± 0.94
Artocarpin (**3**)	0.90 ± 1.63
Morachalcon A (**4**)	0.18 ± 0.10
Acid kojic	3.97 ± 1.45

CONCLUSION

Chemical composition of EtOAc extract of the heartwood of Vietnamese *Artocarpus heterophyllus* has been investigated. Four compounds, cudraflavon B (**1**), brosimon I (**2**), artocarpin (**3**) and morachalcon A (**4**) were isolated and structural elucidated. In addition, they show good inhibition of tyrosinase with the IC_{50} values of 1.03; 1.78; 0.90 and 0.18 μg/mL, respecitvely. This finding suggests that the EtOAc extract of *Artocarpus heterophyllus* could be used as whitening agent for human.

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