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Chemical constituents of the ethyl acetate extract of *Miliusa velutina* Dun Hook. f. et. Thoms.

Hoang-Khang Le[a,b], Thanh-Tung Phan[a,b], Thuy-Duong Ngo Thi[a,b], Tan-Tai Nguyen[a,b], Ngoc-Vinh Huynh[a,b], That- Quang Ton[a,b,*]

[a] *Faculty of Chemistry, University of Science, Ho Chi Minh City, Vietnam*

[b] *Vietnam National University, Ho Chi Minh City, Vietnam*

Abstract

Miliusa velutina Dun Hook. f. et. Thoms is the rare and native plant, belongs to the Annonaceae family. The isolated compounds in *Miliusa* genus showed that many function groups and have diverse bioactivities. From the ethyl acetate extract of the trunk of *Miliusa velutina*, three compounds were isolated and determined based on 1D, 2D NMR spectra, MS and combined comparison with references. The structurally defined compounds are: 4-hydroxybenzoic acid **(1)**, *p*-coumaric acid **(2)**, 1-*O*-methyl-*β*-*D*-glucopyranoside **(3)**. All the three compounds are first known to exist in the *Miliusa velutina*.

Keywords: *Miliusa velutina*, annonaceae, constituents, trunk, phenol.

[*] Corresponding author: E-mail: ttquang@hcmus.edu.vn

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Figure 1. *Miliusa velutina* Dun Hook. f. et. Thoms.

1. Introduction

Miliusa velutina Dun Hook. f. et. Thoms. (**Figure 1**) belongs to the Annonaceae family. Previous biological studies on *Miliusa velutina* reported that its different parts possessed many bioactivities such as nasal sinusitis, uterine inflammation, stomach pain, hemorrhage,... *Miliusa velutina* is also used to treat skin diseases, scabies, ringworms, boils.[1] On the chemical constituent aspect, this species was known to the presence of alkaloids, sesquiterpenes, phenols, steroids, acetogenins,....Those compounds exhibited interesting biological activities.[2-7] To contribute to the chemical constituent knowledge of species, this research reported the isolations and chemical structure elucidation of three compounds from the trunk of *Miliusa velutina* collected in An Giang.

From ethyl acetate extract, three compounds were isolated as follows: 4-hydroxybenzoic acid **(1)**, *p*-coumaric acid **(2),** 1-*O*-methyl-*β*-*D*-glucopyranoside **(3)** (**Figure 2**). The chemical structure of these compounds is determined by modern methods of physicochemical analysis combined with reference comparison.

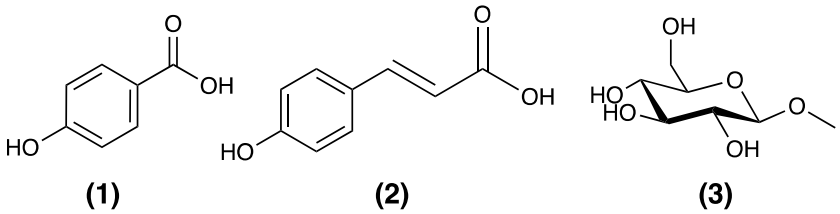

Figure 2. Chemical structures of three isolated compounds

2. Experimental

2.1. Chemicals and equipment

Organic solvents used in column chromatography and thin layer chromatography: *n*-hexane, chloroform, ethyl acetate, methanol, acetone are all chemicals of Chemsol–Vietnam. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} or silica gel 60 RP-18 $F_{254}S$ (Merck). Coloring reagents: H_2SO_4 10% solution; 5% vanillin/ethanol solution. Silica gel column

chromatography phase usually uses silica gel 230-400 mesh RM7484, RP-18 (25-40μm) (Merck). 1D and 2D NMR spectra are recorded on Bruker Avance 500 (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR); HR-ESI-MS data were acquired on Bruker micrOTOF QII (Bruker Singapore Pte., Ltd.) mass spectrometer at Central Laboratory for Analysis, University of Science, Vietnam National University Ho Chi Minh City.

2.2. Plant material

Miliusa velutina sample was collected in An Phu commune, Tinh Bien district, An Giang province in September 2016 and was authenticated by Botanist Hoang Viet, Department of Ecology - Evolutionary Biology, Faculty of Biology – Biotechnology, VNUHCM–University of Science, Ho Chi Minh City. A voucher specimen (MVE 2016) has been deposited at the Department of Organic Chemistry, Faculty of Chemistry, VNUHCM - University of Science.

2.3. Extraction and isolation

Dried trunk of plant powder (10.5 kg) was extracted under reflux successively with *n*-hexane (HE), ethyl acetate (EA), and methanol (ME) to obtain three corresponding extracts: *n*-hexane (45.3 g), ethyl acetate (121.0 g) and methanol (593.7 g). The ethyl acetate extract (121.0 g) was subjected to a silica gel column chromatography and eluted with solvents system of EA:ME (stepwise, 99:1, 98:2, 95:5, 90:10, 80:20, 70:30, 50:50, 0:1) to give 17 fractions (B1-B17). Fraction B5 (619.7 mg) was subjected to a silica gel column chromatography, eluted with solvents system of EA:ME (stepwise, same in the above fraction) to give eight sub-fraction (B5.1-B5.8). Fraction B5.5 (363.1 mg) subjected to a silica gel column chromatography, eluted with solvents system chloroform (CL):ME (stepwise, 99:1, 98:2, 95:5, 90:10, 0:100) to give five sub-fraction (B5.5.1-B5.5.5). Fraction B5.5.4 (50.9 mg) was chromatographed on C18 reversed-phase silica gel column and eluted with ME:water (20:80) to yield compound **1** (9.0 mg) and compound **2** (6.8 mg). Fraction B10 (10.8 g) was subjected to a silica gel column chromatography, eluted with solvents system of EA:ME (stepwise, 80:20, 70:30, 50:50, 0:1) to give night sub-fractions (B10.1-B10.9). Fraction B10.6 (1.6 g) was subjected to a silica gel column chromatography, eluted with solvents system of EA:ME (stepwise, 80:20, 70:30, 50:50, 0:100) to give four sub-fractions (B10.6.1-B10.6.4). Fractions B10.6.3 300 mg was subjected to a silica gel column chromatography, eluted with solvents system of CL:ME (stepwise, 80:20, 70:30, 0:100) to yield compound **3** (49.0 mg).

4-hydroxybenzoic acid (1): white powder. HR-ESI-MS *m/z* 137.0245 $[M-H]^-$ (calcd. for $[C_7H_6O_3-H]^-$, 137.0239). ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 7.77 (2H, *d*, *J*= 8.2 Hz, H-3, H-5), 6.81 (1H, *d*, *J* = 7.4 Hz, H-2, H-6).^{13}C NMR (125 MHz, DMSO-d_6): δ_C 167.6 (C-7), 161.5 (C-4), 131.5 (C-3, C-5), 122.0 (C-1), 115.1 (C-1, C-6).

***p*-Coumaric acid (2)**: yellow oil. HR-ESI-MS *m/z* 163.0397 [M-H]- (calcd. for $[C_9H_8O_3-H]^-$, 163.0395) ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 7.48 (2H, *d*, *J*= 8.4 Hz, H-2, H-6), 6.78 (2H, *d*, *J*= 8.3 Hz, H-3, H-5), 7.45 (1H, *d*, *J*= 16.1 Hz, H-7), 6.27 (1H, *d*, *J*= 16.0 Hz, H-8).^{13}C NMR (125 MHz, DMSO-d_6): δ_C 168.3 (C-9), 159.5 (C-4), 143.5 (C-7), 131.8 (C-2, C-6), 129.9 (C-5), 125.9 (C-1), 115.8 (C-3, C-5), 114.8 (C-8).

1-*O*-methyl-*β*-*D*-glucopyranoside (3): colorless crystal. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 4.16 (1H, *d*, *J*= 7.8 Hz, H-1), 3.87 (1H, *dd*, *J*= 11.8, 1.8 Hz, H-6a), 3.68 (1H, *m*, H-6b), 3.53 (3H, *s*, O-Me). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 105.4 (C-1), 78.1 (C-3), 78.0 (C-5), 75.1 (C-2), 71.7 (C-4), 62.8 (C-6), 57.3 (O-Me).

3. Results and discussion

From the ethyl acetate extract of the trunk of *Miliusa velutina* Dun Hook. f. et. Thoms., three compounds were isolated and determined structures as follows:

Compound **1**: The ^{1}H NMR spectrum showed the presence of two proton signals at δ_H 7.77 (2H, *d*, *J*= 8.2 Hz, H-3, H-5), 6.81 (1H, *d*, *J* = 7.4 Hz, H-2, H-6). The ^{13}C NMR spectrum showed the presence of five carbon signals at δ_C 167.6 (C-7), 161.5 (C-4), 131.5 (C-3, C-5), 122.0 (C-1), 115.1 (C-1, C-6). From the analytical NMR data and comparison with reference[8], compound **1** be concluded as **4-hydroxybenzoic acid**.

Compound **2**: The ^{1}H NMR spectrum showed the presence of four proton signals incluced two proton signals of aromatic protons at δ_H 7.48 (2H, *d*, *J*= 8.4 Hz, H-2, H-6), 6.78 (2H, *d*, *J*= 8.3 Hz, H-3, H-5) and two proton signals of two *trans* olefin protons at 7.45 (1H, *d*, *J*= 16.1 Hz, H-7), 6.27 (1H, *d*, *J*= 16.0 Hz, H-8). The ^{13}C NMR spectrum showed the presence of four carbon signals of aromatic carbons at δ_C 159.5 (C-4), 131.8 (C-2, C-6), 129.9 (C-5), 125.9 (C-1), 115.8 (C-3, C-5), two carbons signals of olefin carbons 143.5 (C-7), 114.8 (C-8), and one carbon signal of carbonyl carbon at 168.3 (C-9).From the analytical NMR data and comparison with reference[9], compound **2** be concluded as ***p*-coumaric acid**.

Compound **3**: The ^{1}H NMR spectrum showed the presence of one proton signals of anomer protons at δ_H 4.16 (1H, *d*, *J*= 7.8 Hz, H-1), one proton signal of *O*-methyl group at 3.53 (3H, *s*, O-Me).. The ^{13}C NMR spectrum showed the presence of six carbon signals incluced one anomer carbone at δ_C 105.4 (C-1), five oxygen carbons signal at 78.1 (C-3), 78.0 (C-5), 75.1 (C-2), 71.7 (C-4), 62.8 (C-6), and one carbon signal of methyl group at 57.3 (O-Me). From the analytical NMR data and comparison with reference[10], compound **3** be concluded as **1-*O*-methyl-*β*-*D*-glucopyranoside**.

4. Conclusions

Studying the chemical composition ethyl acetate extract of the trunks of *Miliusa velutina* using column chromatography techniques combined with thin layer chromatography, the preparation of three pure compounds was established. Based on NMR spectroscopy combined with reference literature comparison, the isolated compounds were: 4-hydroxybenzoic acid **(1)**, *p*-coumaric acid **(2),** 1-*O*-methyl-*β*-*D*-glucopyranoside **(3)**. Three compounds are first known to be present in the *Miliusa velutina* Dun Hook. f. et. Thoms.

Declaration of Competing Interest

The authors declare no competing interests.

Author contributions

Hoang-Khang Le, Thanh-Tung Phan interpreted NMR and MS data and searched the bibliography.

Thuy-Duong Ngo-Thi, Tan-Tai Nguyen, Ngoc-Vinh Huynh, Quang Ton-That contributed to conducting experiments and acquiring MS and NMR data and gave the final correction for the manuscript.

Corresponding author: Quang Ton-That, Faculty of Chemistry, University of Science, Vietnam National University, Ho Chi Minh City, Vietnam, 227 Nguyen Van Cu Street, ward 4, district 5, Ho Chi Minh City, e-mail: ttquang@hcmus.edu.vn.

All authors have read and agreed to the published version of the manuscript.

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