Science & Technology Development Journal, 25(1):2330-2335

Open Access Full Text Article

Research article

Investigation of the *n*-hexane extract of *Melodorum fruticosum* stem

Du Thi Thanh Xuan[1], Nguyen Thi My Huong[1], Nguyen Ngoc Khanh Van[2], Nguyen Kim Phi Phung[3], Nguyen Thi Hoai Thu[4,*]

Use your smartphone to scan this QR code and download this article

ABSTRACT

Introduction: *Melodorum fruticosum* is distributed widely in ASEAN countries. Although this species has been used in folk medicine to cure some diseases, its chemical constituents and biological activity have not been systematically studied. This paper reports the isolation and structural elucidation of seven compounds from the *n*-hexane extract as well as their cytotoxicity against Jurkat and MCF-7-cell lines. **Method:** Dried powder of *Melodorum fruticosum* stem was macerated in ethanol at room temperature to prepare the crude extract. This crude was separated into *n*-hexane, ethyl acetate, and the remaining aqueous extracts by the liquid–liquid partition method. Then, the organic compounds were isolated by chromatographic methods and chemically structurally elucidated by modern spectroscopic techniques. Cytotoxicity assays were performed using the sulforhodamine B method (SBR assay). **Results:** The *n*-hexane extract showed potential activity against the MCF-7-cell line with an IC_{50} value of 14.37 ± 3.18 μg/mL. From this extract, seven compounds consisting of techtochrysin (**1**), pinostrobin (**2**), flavokawin B (**3**), 2′-hydroxy-3′,4′,6′-trimethoxychalcone (**4**), 2′-hydroxy-4′,5′,6′-trimethoxychalcone (**5**), stigmast-4-ene-3-one (**6**), and lupeol (**7**) were isolated and chemically elucidated. **Conclusion:** It is necessary to continue to isolate and perform a bioassay of organic compounds from this extract to determine the bioactive components.

Key words: *Melodorum fruticosum,* flavonoid, chalcone, steroid, terpenoid, cytotoxicity

[1]*Sai Gon University, Ho Chi Minh City, Vietnam*

[2]*Gia Dinh High School, Ho Chi Minh City, Vietnam*

[3]*University of Science, Vietnam National University-Ho Chi Minh City, Ho Chi Minh City, Vietnam*

[4]*University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam*

Correspondence

Nguyen Thi Hoai Thu, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam

Email: nguyenthihoaithu@ump.edu.vn

History

- Received: 2022-02-14
- Accepted: 2022-03-16
- Published: 2022-03-31

DOI : 10.32508/stdj.v25i1.3897

Copyright

© VNUHCM Press. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

INTRODUCTION

Melodorum fruticosum Lour. Merr. (a synonym name, *Rauwenhoffia siamensis* Scheff) belongs to the Annonaceae family. It is distributed in Vietnam, Laos, Cambodia, Thailand, Malaysia, and Indonesia. In Vietnamese folk medicine, leaves of *M. fruticosum* were used to treat digestive ailments and breast swelling, and roots were used to treat abdominal distension and blood tonic for *postpartum women* [1]. Flowers and bark of *M. fruticosum* possess antiviral, antioxidant, and cytotoxic activities [2]. Some extracts and compounds isolated from *M. fruticosum* revealed significant cytotoxicity against some cancer cell lines, such as A-549, MCF-7, HT-29, SK-MEL-5, Malme-3 M, 9-PS, and KBMRI [3,4]. Previously, phytochemical studies reported the presence of flavonoids, alkaloids, steroids, heptenoids, and tannins from leaves, stem bark, and flowers of *M. fruticosum* [3–6]. This paper showed the cytotoxicity against the Jurkat and MCF-7 cancer cell lines of the *n*-hexane extract of the *M. fruticosum* stem and the chemical elucidation of some compounds isolated from this one.

MATERIALS AND METHODS

General experimental procedures

The NMR spectra were registered on Bruker Avance at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. HR-ESI-MS spectra were recorded on a Bruker MicrOTOF-QII. The optical specific rotation was measured on a Kruss (Germany) polarimeter with a tube length of 0.5 decimetres.

Plant material

The stem of *Melodorum fruticosum* L. was collected at Di Linh Ward, Lam Dong Province, Vietnam, in July 2017. The scientific name of this species was authenticated by Dr. Dang Van Son, Institute of Tropical Biology, Vietnam Academy of Science and Technology. A voucher specimen (N^{o} US-A012) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, Vietnam National University - Ho Chi Minh City.

Extraction and isolation

The dried powder of *M. fruticosum* stem (90 kg) was macerated in ethanol at room temperature, and then the filtrated solution was evaporated at reduced pressure to give a crude extract (6 kg). The liquid–liquid

Cite this article : Xuan D T T, Huong N T M, Van N N K, Phung N K P, Thu N T H. **Investigation of the *n*-hexane extract of *Melodorum fruticosum* stem**. *Sci. Tech. Dev. J.;* 25(1):2330-2335.

partition method with *n*-hexane and ethyl acetate, in turn, was used to separate the crude extract into different fractions.

The *n*-hexane extract (250 g) was subjected to normal-phase silica gel column chromatography. The eluent solvent systems *n*-hexane:ethyl acetate (stepwise, 99:1, 98:2, 95:5, 90:10, 80:20, 50:50, 0:100) were used to afford 11 fractions (H1–H11). Fraction H3 (20.0 g) was further separated into 6 subfractions (H3.1–H3.6) by normal-phase silica gel column chromatography eluted with *n*-hexane:ethyl acetate (stepwise, 95:5, 90:10, 80:20). Subfraction H3.2 (4.48 g) was subjected to silica gel column chromatography and eluted with solvent systems of *n*-hexane:ethyl acetate (95:5, 90:10, in turn) to give compound **1** (10.0 mg). The same method was applied to subfraction H3.3 (2.58 g) to afford **3** (8.9 mg) and **5** (7.0 mg). A silica gel column chromatography eluted with *n*-hexane:ethyl acetate (95:5, 90:10, in turn) was applied to subfraction H3.5 (4.36 g) to afford four subfractions (H3.5.1–H3.5.4). Compounds **6** (7.0 mg) and **7** (9.6 mg) were isolated from subfraction H3.5.1 (0.20 g), while compound **2** (12.0 mg) was obtained from subfraction H3.5.3 (1.02 g) via silica gel column chromatography eluted with *n*-hexane:ethyl acetate (stepwise, 95:5, 90:10). Finally, compound **4** (7.4 mg) was isolated from fraction H3.6 (4.05 g) through silica gel column chromatography, eluted with *n*-hexane:ethyl acetate (95:5, 90:10, in turn), further purified by Sephadex LH-20, and eluted with methanol.

Cytotoxic assay

The cytotoxicity activities against Jurkat and MCF-7 cancer cell lines of the *n*-hexane extract and compounds **1** and **2**, using the sulforhodamine B method (SBR assay) and camptothecin as a positive control, were determined as described in a previous report[7]. This assay was performed at the Molecular Biology Laboratory, Faculty of Biology, University of Science. Vietnam National University-Ho Chi Minh City.

RESULTS

From the *n*-hexane extract of *Melodorum fruticosum* collected in Di Linh Ward, Lam Dong Province, seven compounds, **1** (10.0 mg), **2** (12.0 mg), **3** (8.9 mg), **4** (7.4 mg), **5** (7.0 mg), **6** (7.0 mg), and **7** (9.6 mg), were isolated. Their physical properties and spectroscopic data were obtained as follows.

Techtochrysin (1): Yellow powder. HR-ESI-MS: *m/z* 291.0628 [M+Na]$^+$ (calcd. for $C_{16}H_{12}O_4Na$, 291.0633). ^{1}H-NMR data (DMSO-d_6) (*J* in Hertz): δ_H 7.03 (*s*, H-3), 6.40 (*d*, 2.0, H-6), 6.82 (*d*, 2.0, H-8), 8.11 (*dd*, 8.0, 1.5, H-2′/H-6′), 7.59 (*m*, H-3′/H-5′), 7.62 (*m*, H-4′), 3.88 (*s*, 7-OCH_3), and 12.81 (*s*, 5-OH). ^{13}C-NMR (DMSO-d_6): δ_C 163.5 (C-2), 105.3 (C-3), 182.0 (C-4), 161.1 (C-5), 98.1 (C-6), 156.1 (C-7), 92.8 (C-8), 157.4 (C-9), 104.9 (C-10), 130.6 (C-1′), 126.4 (C-2′/C-6′), 129.1 (C-3′/C-5′), 132.1 (C-4′) and 56.1 (7-OCH_3). Selected HMBC correlations: see Figure 1.

Pinostrobin (2): Yellow powder. $[\alpha]_D^{25}$ 0 (*c* 0.10, acetone) (Pinostrobin, not mentioned)[9]. HR-ESI-MS: *m/z* 293.0784 [M+Na]$^+$ (calcd. for $C_{16}H_{14}O_4Na$, 293.0790). ^{1}H-NMR data (DMSO-d_6) (δ_H, *J* in Hertz): 5.63 (*dd*, 13.0, 3.0, H-2), 2.83 (*dd*, 17.0, 3.0, H-3a), 3.31 (*dd*, 17.0, 13.0, H-3b), 6.10 (*d*, 2.5, H-6), 6.15 (*d*, 2.5, H-8), 7.52 (*dd*, 8.5, 1.0, H-2′/H-6′), 7.43 (*m*, H-3′/H-5′), 7.39 (*m*, H-4′), 3.80 (*s*, 7-OCH_3), and 12.10 (*s*, 5-OH). ^{13}C-NMR data (DMSO-d_6): δ_C 78.5 (C-2), 42.1 (C-3), 196.4 (C-4), 163.2 (C-5), 94.7 (C-6), 167.4 (C-7), 93.8 (C-8), 162.6 (C-9), 102.6 (C-10), 138.5 (C-1′), 126.6 (C-2′/C-6′), 128.5 (C-3′/C-5′), 128.5 (C-4′) and 55.8 (7-OCH_3). Selected HMBC correlations: see Figure 1.

Flavokawin B (3): Yellow powder. HR-ESI-MS: *m/z* 285.1180 [M+H]$^+$ (calcd. for $C_{17}H_{16}O_4$+H, 285.1127). ^{1}H-NMR data ($CDCl_3$) (δ_H, *J* in Hertz): 7.61 (*dd*, 8.0, 1.5, H-2/H-6), 7.41 (*m*, H-3/H-5), 7.40 (*m*, H-4), 7.74 (*d*, 16.0, H-7), 7.90 (*d*, 16.0, H-8), 6.11 (*d*, 2.5, H-3′), 5.97 (*d*, 2.5, H-5′), 3.92 (*s*, 4′-OCH_3), and 3.84 (*s*, 6′-OCH_3). ^{13}C-NMR data ($CDCl_3$): δ_C 135.6 (C-1), 128.4 (C-2/C-6), 128.9 (C-3/C-5), 130.1 (C-4), 142.3 (C-7), 127.6 (C-8), 192.7 (C-9), 106.4 (C-1′), 166.3 (C-2′), 93.9 (C-3′), 168.4 (C-4′), 91.3 (C-5′), 162.6 (C-6′), 55.9 (4′-OCH_3) and 55.6 (6′-OCH_3). Selected HMBC correlations: see Figure 1.

2′-Hydroxy-3′,4′,6′ -trimethoxychalcone (4): Yellow powder. HR-ESI-MS: *m/z* 337.1054 [M+Na]$^+$ (calcd. for $C_{18}H_{18}O_5Na$, 337.1052). ^{1}H-NMR data (DMSO-d_6) (δ_H, *J* in Hertz): 7.72 (*dd*, 80, 1.5, H-2/H-6), 7.45 (*m*, H-3/H-5), 7.44 (*m*, H-4), 7.63 (*d*, 16.0, H-7), 7.66 (*d*, 16.0, H-8), 12.63 (*s*, 2′-OH), 6.31 (*s*, H-5′), 3.65 (*s*, 3′-OCH_3), and 3.92 (*s*, 4′-OCH_3/6′-OCH_3). ^{13}C-NMR data (DMSO-d_6): δ_C 134.7 (C-1), 128.4 (C-2/C-6), 128.9 (C-3/C-5), 134.7 (C-4), 142.4 (C-7), 127.6 (C-8), 192.7 (C-9), 107.1 (C-1′), 157.7 (C-2′), 130.0 (C-3′), 157.6 (C-4′), 88.5 (C-5′), 157.2 (C-6′), 60.0 (3′-OCH_3), 56.3 (4′-OCH_3) and 56.0 (6′-OCH_3). Selected HMBC correlations: see Figure 1.

2′-Hydroxy-4′,5′,6′ -trimethoxychalcone (5): Yellow powder. HR-ESI-MS: *m/z* 337.1048 [M+Na]$^+$ (calcd. for $C_{18}H_{18}O_5Na$, 337.1052). ^{1}H-NMR data ($CDCl_3$) (δ_H, *J* in Hertz): 7.64 (*dd*, 8.0, 1.5, H-2/H-6), 7.43 (*m*, H-3/H-5), 7.39 (*m*, H-4), 7.84 (*d*, 15.5, H-7),

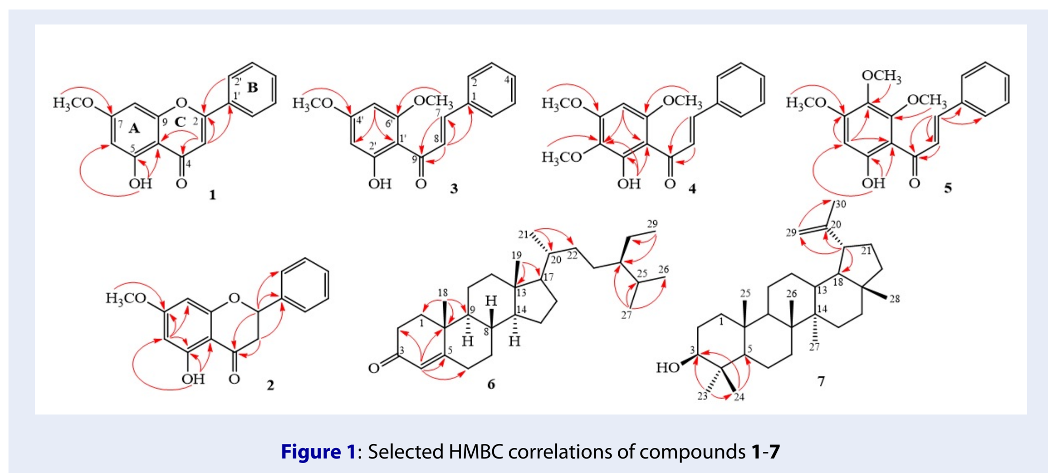

Figure 1: Selected HMBC correlations of compounds **1**-**7**

7.93 (*d*, 15.5, H-8), 13.64 (*s*, 2′-OH), 6.29 (*s*, H-3′), 3.84 (*s*, 4′-OCH_3), 3.90 (*s*, 4′-OCH_3), and 3.94 (*s*, 6′-OCH_3). ^{13}C-NMR data ($CDCl_3$): δ_C 135.3 (C-1), 128.4 (C-2/C-6), 129.0 (C-3/C-5), 130.3 (C-4), 143.2 (C-7), 126.6 (C-8), 193.0 (C-9), 108.8 (C-1′), 162.7 (C-2′), 96.6 (C-3′), 155.0 (C-4′), 135.4 (C-5′), 160.2 (C-6′), 61.9 (4′-OCH_3), 61.3 (5′-OCH_3), and 56.1 (6′-OCH_3). Selected HMBC correlations: see Figure 1.

Stigmast-4-ene-3-one (6): White powder. HR-ESI-MS: *m/z* 435.3555 $[M+Na]^+$ (calcd. for $C_{29}H_{48}ONa$, 435.3603). ^{1}H-NMR data ($CDCl_3$) (δ_H, *J* in Hertz): 5.72 (*s*, H-4), 0.71 (*s*, H-18), 1.18 (*s*, H-19), 0.92 (*d*, 6.5, H-21), 0.84 (*d*, 7.5, H-26), 0.81 (*d*, 7.5, H-27), and 0.85 (*t*, 7.5, H-29). ^{13}C-NMR data ($CDCl_3$): δ_C 35.7 (C-1), 34.0 (C-2), 199.6 (C-3), 123.7 (C-4), 171.8 (C-5), 33.0 (C-6), 32.1 (C-7), 35.7 (C-8), 53.9 (C-9), 38.6 (C-10), 21.0 (C-11), 39.7 (C-12), 42.4 (C-13), 56.0 (C-14), 24.2 (C-15), 28.2 (C-16), 56.0 (C-17), 12.0 (C-18), 17.4 (C-19), 36.1 (C-20), 18.7 (C-21), 34.0 (C-22), 26.1 (C-23), 45.9 (C-24), 29.2 (C-25), 20.0 (C-26), 19.0 (C-27), 23.1 (C-28), and 11.9 (C-29). Selected HMBC correlations: see Figure 1.

Lupeol (7): White powder. ^{1}H-NMR data ($CDCl_3$) (δ_H, *J* in Hertz): δ_H 3.19 (*dd*, 11.5, 5.0, H-3), 2.39 (*ddd*, 11.5, 11.5, 6.9, H-19), 0.97 (*s*, H-23), 0.76 (*s*, H-24), 0.83 (*s*, H-25), 1.03 (*s*, H-26), 0.94 (*s*, H-27), 0.79 (*s*, H-28), 4.68 (*d*, 2.5, H-29a), 4.56 (*m*, H-29b), and 1.68 (*brs*, H-30). ^{13}C-NMR data ($CDCl_3$): δ_C 38.8 (C-1), 27.5 (C-2), 79.0 (C-3), 38.9 (C-4), 55.4 (C-5), 18.4 (C-6), 34.3 (C-7), 41.0 (C-8), 50.5 (C-9), 37.2 (C-10), 21.0 (C-11), 25.2 (C-12), 38.1 (C-13), 42.8 (C-14), 27.5 (C-15), 35.6 (C-16), 43.2 (C-17), 48.3 (C-18), 48.2 (C-19), 151.0 (C-20), 29.9 (C-21), 40.0 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 16.0 (C-26), 14.6 (C-27), 18.0 (C-28), 109.3 (C-29), and 19.3 (C-30). Selected HMBC correlations: see Figure 1.

DISCUSSION

Compound **1** was isolated as a yellow powder. The high-resolution mass spectrum of **1** showed a sodiated molecular ion peak at *m/z* 291.0628 $[M+Na]^+$ (calcd. for $C_{16}H_{12}O_4Na$, 291.0633). The proton spectrum of **1** displayed a singlet signal at δ_H 12.81 of the hydroxy group at C-5 possessing an intramolecular hydrogen bond with the carbonyl group at C-4 in the flavonoid skeleton, as usual. Two doublet signals at δ_H 6.40 and 6.82 with the small coupling constant of 2.0 Hz were assigned to H-6 and H-8, respectively, of ring A. One singlet aromatic proton signal at δ_H 7.03 (*s*, H-3) was H-3 of ring C. Five aromatic protons at δ_H 8.11 (*dd*, 8.0, 1.5 Hz, H-2′/H-6′), 7.59 (*m*, H-3′/H-5′), and 7.62 (*m*, H-4′) belonged to a phenyl group (ring C). In addition, at a high magnetic field, there was a singlet methoxy proton signal at δ_H 3.88 (*s*, 7-OCH_3). This information suggested that **1** was a flavone possessing a hydroxy group at C-5 and a methoxy group at C-7. This corresponded to the presence of fourteen carbon signals, including one methoxy carbon, two aromatic carbons at δ_C 126.4 (C-2′/C-6′) and 129.1 (C-3′/C-5′), appearing in double intensity and possessing both HSQC and HMBC experiments with protons H-2′/H-6′ and H-3′/H-5′, respectively, of a symmetrical benzene B ring. This phenyl group, attached to C-2, was determined by the HMBC cross-peaks of the proton H-3 to carbon C-1′ (δ_C 130.6) and of protons H-2′/H-6′ to carbons C-2 and C-1′ (Figure 1). The HMBC spectrum also revealed the cross-peaks of the hydroxy proton with carbons C-5, C-6, and C-10 and of the methoxy proton with carbon at δ_C 156.1 (C-7). Based on the compatibility of the NMR data of **1** with those in the literature[8], **1** was assigned to be techtochrysin.

Compound **2** was isolated as a yellow powder. The HR-ESI-MS spectrum of **2** displayed a quasi-molecular ion peak at *m/z* 293.0784 $[M+Na]^+$ (calcd. for $C_{16}H_{14}O_4Na$, 293.0790). The comparison of the NMR data of **1** and **2** showed that carbons C-2 and C-3 of **2** were saturated, which was confirmed by the presence of two methylene proton signals at δ_H 2.83 (*dd*, 17.0, 3.0 Hz, H-3a) and 3.31 (*dd*, 17.0, 13.0 Hz, H-3b) and one oxygenated methine proton at δ_H 5.63 (*dd*, 13.0, 3.0 Hz, H-2), instead of the singlet olefinic proton H-3 as in **1**. This corresponded to the observation of one methylene carbon signal at δ_C 42.1 (C-3) and one oxygenated methine at δ_C 78.5 (C-2), where its HSQC spectrum showed correlations to protons H-3 and H-2, respectively. This feature was further confirmed by the HMBC cross-peaks of both protons H-2 and H-3 to a carbonyl carbon at δ_C 196.4 (C-4) and of proton H-2 to carbons C-1′ and C-2′/C-6′. The positions of the two substituents (-OH and -OCH_3) were confirmed by the HMBC correlations, as shown in Figure 1. Due to the null specific optical rotation and the good compatibility of NMR data of **2** with published data [9], **2** was thus determined to be a racemic mixture of pinostrobin.

Compound **3** was isolated as a yellow powder. Its molecular formula was determined to be $C_{17}H_{16}O_4$ based on the protonated molecular ion peak at *m/z* 285.1180 $[M+H]^+$ (calcd. for $C_{17}H_{16}O_4$+H, 285.1127). Similar to **1**, the proton spectrum of **3** revealed two doublet signals with small coupling constants at δ_H 6.11 (*d*, 2.5 Hz, H-3′) and 5.97 (*d*, 2.5 Hz, H-5′) of ring A, signals at δ_H 7.61 (*dd*, 8.0, 1.5 Hz, H-2/H-6), 7.41 (*m*, H-3/H-5), and 7.40 (*m*, H-4) of ring B. However, in comparison to the NMR data of **1,** instead of an olefinic methine as in **1** (δ_H 7.03, δ_C 105.3, C-3), the NMR spectra of **3** showed one more methoxy signal and two olefinic carbons in the *E*-configuration [(δ_H 7.74, *d*, 16.0 Hz, δ_C 142.3, C-7) and 7.90 (*d*, 16.0 Hz, δ_C 127.6, C-8)]. These features showed that **3** would be a chalcone. This was in accordance with the unsaturated degree value of 10 in **3** calculated from the molecular formula of $C_{17}H_{16}O_4$ as well as the HMBC cross-peaks of protons of the *trans*-double bond with the carbonyl carbon C-9 (δ_C 192.7) and the quaternary carbon C-1 (δ_C 135.6) of the B ring. Finally, **3** was elucidated as flavokawin B by the good compatibility of its NMR data with those of flavokawin B [10].

Compound **4** was isolated as a yellow powder. The molecular formula of **4** was identified to be $C_{18}H_{18}O_5$ based on the pseudomolecular ion peak at *m/z* 337.1054 $[M+Na]^+$ (calcd. for $C_{18}H_{18}O_5Na$, 337.1052) in the HR-ESI-MS. The similarity in the NMR data of **4** and **3** with just one difference of the replacement of the aromatic methine proton in **3** by a methoxy group in **4**. The comparison of NMR data of **4** with the 2′-hydroxy-3′,4′,6′-trimethoxychalcone in the literature was compatible [11]. The positions of the hydroxy and three methoxy groups were supported by HMBC experiments, as presented in Figure 1. Therefore, the chemical structure of **4** was as shown.

Compound **5** was isolated as a yellow powder. The HR-ESI-MS spectrum of **5** showed a pseudomolecular ion peak at *m/z* 337.1048 $[M+Na]^+$ (calcd. for $C_{18}H_{18}O_5Na$, 337.1052); therefore, the molecular formula of **5** was determined to be $C_{18}H_{18}O_5$. Although two compounds **4** and **5** were isolated from two different fractions, they possessed the same molecular formula of $C_{18}H_{18}O_5$ and similar chemical shift values therefore, **5** should be a positional isomer of **4**. The comparison of NMR data of **5** with 2′-hydroxy-4′,5′,6′-trimethoxychalcone [12] showed good compatibility. Its HMBC experiment well supported the proposed structure with cross-peaks of the hydroxy proton at δ_H 13.64 (*s*, 2′-OH) to carbons at δ_C 108.8 (C-1′), 162.7 (C-2′), and 96.6 (C-3′), of the methine proton at δ_H 6.29 (*s*, H-3′) to carbons at δ_C 108.8 (C-1′), 155.0 (C-4′), and 135.4 (C-5′), and of three methoxy protons with carbons C-4′, C-5′ and C-6′. Therefore, **5** was determined to be 2′-hydroxy-4′,5′,6′-trimethoxychalcone.

Compound **6** was isolated as a white powder. The molecular formula of **6** was determined to be $C_{29}H_{48}O$ via the sodiated molecular ion peak at *m/z* 435.3555 $[M+Na]^+$ (calcd. for $C_{29}H_{48}ONa$, 435.3603). The ^{13}C-NMR spectrum of **6** showed signals of 29 carbons, consisting of one carbonyl carbon at δ_C 199.6 (C-3) and two olefinic carbons at δ_C 123.7 (C-4) and 171.8 (C-5) of a conjugated ketone system. The remaining 26 carbons resonated at a high magnetic field from 11 to 56 ppm. These results suggested that **6** could be a sterol possessing a conjugated ketone group (>C=CH–CO–). Its ^{1}H-NMR spectrum with two singlets, three doublets, and a triplet signal from 0.7 to 1.2 ppm was characterized for a stigmastane skeleton. In addition, the sole signal that resonated at the low magnetic field at δ_H 5.72 (*s*) belonged to the olefinic proton. The positions of two olefinic carbons, C-4 and C-5, were determined by the HMBC cross-peaks of the olefinic proton to carbons at δ_C 34.0 (C-2), 171.8 (C-5), 33.0 (C-6), and 38.6 (C-10). This meant that the position of the conjugated carbonyl carbon was suggested to be at C-3. In the comparison of the NMR data of **6** with published data [13], **6** was thus determined to be stigmast-4-ene-3-one.

Compound **7** was isolated as a white powder. The ^{1}H and ^{13}C-NMR data analysis of **7** suggested that it possessed a lupane skeleton characterized by signals of an isopropenyl unit at δ_H 4.68 (1H, *d*, 2.5 Hz, H-29a), 4.56 (1H, *m*, H-29b), and 1.68 (3H, *brs*, H-30) in the proton spectrum and two olefinic carbon signals at δ_C 151.0 (C-20) and 109.3 (C-29) in the ^{13}C-NMR spectrum. In addition, 7 had a hydroxy group at C-3 as usual, which was confirmed by the HMBC cross-peaks of two singlet methyl proton signals at δ_H 0.97 (s, H-23) and 0.76 (s, H-24) to carbon C-3. Compound **7** was determined to be lupeol via the good compatibility of its NMR data with those published in the literature[14].

The *n*-hexane extract and compounds **1** and **2** were evaluated for their cytotoxicity against Jurkat and MCF-7 cancer cell lines. The results showed that the *n*-hexane extract possessed significant cytotoxicity against the MCF-7 cancer cell line with an IC_{50} value of 14.37 ± 3.18 μg/mL. However, compounds **1** and **2** revealed weak activities against the two surveyed cancer cell lines. Therefore, the components that induced the toxicity of the *n*-hexane extract could be from other compounds in this extract. The isolation and bioassay of organic compounds from this extract should be continued.

CONCLUSION

From the *n*-hexane extract of *Melodorum fruticosum* stem, seven compounds were isolated consisting of two flavonoids (techtochrysin and pinostrobin), three chalcones (flavokawin B, 2′-hydroxy-3′,4′,6′-trimethoxychalcone, and 2′-hydroxy-4′,5′,6′-trimethoxychalcone), a steroid (stigmast-4-ene-3-one), and a triterpenoid (lupeol). Their chemical structures were elucidated based on NMR and HR-MS data analysis as well as comparison to published data. The *n*-hexane extract showed significant cytotoxicity against the MCF-7-cell line with an IC_{50} value of 14.37 ± 3.18 μg/mL, whereas compounds **1** and **2** isolated from this extract were inactive. To the best of our knowledge, except for **1**, six remaining compounds were isolated from *Melodorum fruticosum* for the first time.

ABREVIATIONS

HR-ESI-MS: High resolution electrospray ionization-Mass spectrometry
^{1} H-NMR: Proton nuclear magnetic resonance
^{13} C-NMR: Carbon-13 nuclear magnetic resonance
HSQC: Heteronuclear single quantum coherence
HMBC: Heteronuclear multiple bond correlation
s: singlet
brs: broad singlet
d: doublet
dd: doublet of doublets
ddd: doublet of doublet of doublets
t: triplet
m: multiplet
calcd.: calculated
IC_{50}: Half-maximal inhibitory concentration
Jurkat cell: immortalized T-lymphocyte cell line
MCF-7 cell: breast cancer cell line

COMPETING INTEREST

The authors declare no competing financial interest.

AUTHORS' CONTRIBUTION

Du T.T.X. and Nguyen T.M.H. contributed to conducting experiments and acquiring data. Nguyen N.K.V. and Nguyen K.P.P. interpreted NMR and MS data. Nguyen T.H.T. provided final approval of the manuscript to be submitted.

REFERENCES

1. Pham HH. Vietnamese plants. Ho Chi Minh City Young Publisher. 1999; 246;.
2. Chaichantipyuth C, Tiyaworanan S, Mekaroonreung S, Ngamrojnavanich N, Roengsumran S, Puthong S, Petsom A, Ishikawa T. Oxidized heptenes from flowers of Melodorum fruticosum. Phytochemistry. 2001; 58:1311-1315;Available from: https://doi.org/10.1016/S0031-9422(01)00215-1.
3. Jung JH, Pummangura S, Chaichantiputh C, Patarapanich C, Mclaughlin JL. Bioactive constituents of Melodorum fruticosum. Phytochemistry. 1990; 29(5):1667-1670;Available from: https://doi.org/10.1016/0031-9422(90)80142-4.
4. Jung JH, Chang CJ, Smith DL, Mclaughlin JL, Pummangura S, Chaichantipyuth C, Patarapanich C. 1991, Additional bioactive heptenes from Melodorum fruticosum. J Nat Prod. 1991; 54(2):500-505;Available from: https://doi.org/10.1021/np50074a023.
5. Mongkol R, Piapukiew J, Chavasiri W. Chemical constituents from Melodorum fruticosum Lour. flowers against plant pathogenic fungi. Agric Nat Resour. 2016; 50(4):270-275;Available from: https://doi.org/10.1016/j.anres.2016.03.001.
6. Chan HH, Hwang TL, Thang TD, Leu YL, Kuo PC, Nguyet BTM, Dai DN, Wu TS. Isolation and synthesis of melodamide A, a new anti-inflammatory phenolic amide from the leaves of Melodorum fruticosum, Planta Med. 2013; 79:288-294;Available from: https://doi.org/10.1055/s-0032-1328131.
7. Nguyen THT, Pham HVT, Pham NKT, Quach NDP, Pudhom K, Hansen PE, Nguyen KPP. Chemical constituents from Sonneratia ovata Backer and their in vitro cytotoxicity and acetylcholinesterase inhibitory activities. Bioorg Med Chem Lett. 2015; 25(11):2366-2371;Available from: https://doi.org/10.1016/j.bmcl.2015.04.017.
8. Hasan MM, Ahmed QU, Soad SZM, Latip J, Taher M, Syafiq TMF, Sarian MN, Alhassan AM, Zakaria ZA. Flavonoids from Tetracera indica Merr. induce adipogenesis and exert glucose uptake activities in 3T3-L1 adipocyte cells. BMC Complement Altern Med. 2017; 17:431;Available from: https://doi.org/10.1186/s12906-017-1929-3.
9. Smolarz HD, Mendyk E, Bogucka-Kocka A, Kockic J, Pinostrobin - an anti-leukemic flavonoid from Polygonum lapathifolium L. ssp. nodosum (Pers.) Dans, Z Naturforsch. 2006; 61c:64-68;Available from: https://doi.org/10.1515/znc-2006-1-212.

10. Derita M, Zacchino S. Chemotaxonomic importance of sesquiterpenes and flavonoids in five Argentinian species of Polygonum genus. J Essent Oil Res. 2011; 23:11-14;Available from: https://doi.org/10.1080/10412905.2011.9700474.
11. Panichpol K, Waterman PG. Novel flavonoids from the stem of Popowia cauliflora. Phytochemistry. 1978; 17:1363-1367;Available from: https://doi.org/10.1016/S0031-9422(00)94590-4.
12. Tan CX, Schrader KK, Khan IA, Rimando AM. Activities of wogonin analogs and other flavones against Flavobacterium columnare. Chem Biodivers. 2015; 12(2):259-272;Available from: https://doi.org/10.1002/cbdv.201400181.
13. Gaspar EMM, Neves HJC das. Steroidal constituents from mature wheat straw. Phytochemistry. 1993; 34(2):523-527;Available from: https://doi.org/10.1016/0031-9422(93)80039-U.
14. Na M, Kim BY, Osada H, Ahn JS. 2009, Inhibition of protein tyrosine phosphatase 1B by lupeol and lupenone isolated from Sorbus commixta. J Enzyme Inhib Med Chem. 2009; 24(4):1056-1059;Available from: https://doi.org/10.1080/14756360802693312.

Tạp chí Phát triển Khoa học và Công nghệ
Đại học Quốc gia Tp. Hồ Chí Minh

Tạp chí Phát triển Khoa học và Công nghệ
ISSN: 1859-0128
Hình thức xuất bản: In và trực tuyến
Hình thức truy cập: Truy cập mở (Open Access)
Ngôn ngữ bài báo: Tiếng Anh
Tỉ lệ chấp nhận đăng 2021: 72%
Phí xuất bản: Miễn phí
Thời gian phản biện: 43 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Tự nhiên
ISSN: 2588-106X
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 75%
Phí xuất bản: Miễn phí
Thời gian phản biện: 30-45 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ - Kĩ thuật và Công nghệ
ISSN: 2615-9872
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 61%
Phí xuất bản: Miễn phí
Thời gian phản biện: 50 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ - Kinh tế-Luật và Quản lý
ISSN: 2588-1051
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 65%
Phí xuất bản: Miễn phí
Thời gian phản biện: 45 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Xã hội và Nhân văn
ISSN: 2588-1043
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 62%
Phí xuất bản: Miễn thu phí đối với tác giả là CBVC của ĐHKHXHNV, ĐHQG-HCM; Tác giả khác: 500.000 VNĐ/bài
Thời gian phản biện: 75 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Trái đất và Môi trường
ISSN: **2588-1078**
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt và tiếng Anh
Tỉ lệ chấp nhận đăng 2021: 87%
Phí xuất bản: liên hệ tòa soạn
Thời gian phản biện: 45 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Sức khỏe
ISSN: 2734-9446
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 70%
Phí xuất bản: Miễn phí
Thời gian phản biện: 30 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ, Đại học Quốc gia Tp.HCM
25 năm xuất bản học thuật (1997-2022)
Tòa soạn: Nhà điều hành Đại học Quốc gia Tp.HCM, P. Linh Trung, TP. Thủ Đức, TP. HCM
Email: stdj@vnuhcm.edu.vn; tcptkhcn@vnuhcm.edu.vn; Website: http://www.scienceandtechnology.com.vn