Chemical constituents of the lichen *Dendriscosticta platyphylloides*, Lobariaceae

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ABSTRACT

Introduction: Dendriscosticta is a new genus belong the Sticta wrightii group of the family Lobariaceae. This genus of foliose lichen is widely distributed in tropical regions worldwide. The lichen Dendriscosticta platyphylloides is very abundant in Bidoup forest, Lam Dong province, Vietnam. **Methods:** The structure of these compounds was elucidated through the interpretation of their 1D and 2D-NMR and HR-MS data. The cytotoxic activities of these compounds against liver hepatocellular carcinoma (HepG2), human lung cancer (NCI-H460), human epithelial carcinoma (HeLa) and human breast cancer (MCF-7) cell lines was performed at the concentration of 100 μ g/mL using the sulforhodamine B (SRB) assay. **Results:** In this paper, we reported the isolation of six known compounds, including (1) 15 α -acetoxyhopan-22-ol (2) hopane-15 α ,22-diol, (3) zeorin, (4) cerevisterol, (5) salvigenin, and (6) 5-hydroxy-3',4',7-trimethoxyflavone. **Conclusion:** This is the first time that these compounds are isolated from *Dendriscosticta* genus. These compounds showed no cytotoxic activity against four cell lines.

Key words: Dendriscosticta platyphylloides, lichen, hopane, flavone

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INTRODUCTION

The word "lichen" has a Greek origin, which was referred to the superficial growth of fungus like organism on the bark of olive trees. Theophrastus, the Father of Botany coined the term "lichen" during 300 BC and introduced this group of plants to the scientific world¹. Historically, lichens have had economic benefits. Lichens have been used for food, dye, brewing, leather industries, and most importantly, they have been used in folk medicine. Medicinal uses of lichens include treatment of fever, epilepsy, coughing, tuberculosis, rabies, gout, external wounds and hepatitis¹⁻³. Besides, lichens in tropical Vietnamese habitats have not been well chemically studied. In the course of our systematic research on lichen substances from the Vietnamese flora, we have examined Dendriscosticta platyphylloides which is widely distributed in the southwestern part of the central of Vietnam and has not yet been chemically and biologically studied. According to Rober Lucking⁴, Dendriscosticta is a new genus belong the Sticta wrightii group within Lobaria clade. The primary goal of the present work was to study chemical constituent of the lichen Dendriscosticta platyphylloides (Nyl.) Moncada & Lücking.

METHODS

General Experimental Procedures

The NMR spectra were measured on a Bruker Avance III spectrometer (500 MHz for ¹H and 125 MHz for ¹³C), Bruker 400 Avance spectrometer (400 MHz for ¹H and 100 MHz for ¹³C). CDCl₃ and DMSO-*d*₆ were used both as a solvent and as an internal reference at δ_H 7.26, 2.50 and δ_C 77.2, 39.5. The HRESIMS were obtained using a Bruker microOTOF Q-II. TLC was carried out on precoated silica gel 60 F254 or silica gel 60 RP–18 F254S (Merck Millipore, Billerica, Massachusetts, USA). Gravity column chromatography was performed with silica gel 60 (0.040–0.063 mm)(HiMedia, Mumbai, India).

Lichen Material

Thalli of the studied lichen were separated from bark of various old trees in Bidoup Nui Ba National Park, Dam Rong district, Lam Dong province, Vietnam in July – August 2012 **Figure 1**. The species was authenticated as *Dendriscosticta platyphylloides* (Nyl.) by Dr. Robert Lücking (Department of Botany. The Field Museum. Illinois USA). A voucher specimen (No US-B035) was deposited at the Herbarium of the Department of Organic Chemistry, University of Science, National University — Ho Chi Minh City — Vietnam.

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Figure 1: Dendriscosticta platyphylloides (Nyl.).

Extraction and isolation

The fresh lichen thalli were carefully inspected for contaminants, cleaned under running tap water and air-dried. The ground powder sample (2.1 kg) was extracted with EtOH (4 x 15L) at room temperature by the maceration method at ambient temperature, and the filtered solution was evaporated under reduced pressure to afford an EtOH extract (200.0 g). This crude extract (200.0 g) was separated by quick column chromatography, first eluted with *n*-hexane to afford the *n*-hexane fraction (10.6 g), then with a gradient of EtOAc and MeOH (stepwise, 10:0, 9:1, 8:2, 7:3, and 5:5) to afford five EtOAc fractions EAS-1 (29.2 g), EAS-2 (3.1 g), EAS-3 (9.2 g), EAS-4 (14.8 g), and EAS-5 (75.1 g); finally, with MeOH to afford the MeOH fraction M (24.5 g). Fraction EAS-1 was applied to silica gel column chromatography eluted with *n*-hexane-EtOAc (stepwise, 9:1, and 8:2) to give (1). The same manner was applied to fraction EAS-2, eluted with n-hexane-EtOAc (stepwise, 9:1, and 8:2) to give two compounds (3) (63 mg), and (4) (14 mg), to the fraction EAS-3, eluted with *n*-hexane-EtOAc (stepwise, 8:2, 7:3, and 1:1) to afford three compounds (2) (780 mg), (5) (12 mg), and (6) (4 mg).

Cytotoxic assay

Determination of cytotoxic activities against the HeLa (human epithelial carcinoma), MCF–7 (human breast cancer), HepG2 (human hepatoma cancer) and NCI-H460 (human lung cancer) cell lines of isolated compounds was performed at the concentration of 100 mg/mL using the sulforhodamine B (SRB) assay with camptothecin as the positive control.⁵ The details were similar to those presented in our previous paper.⁶

RESULTS

Structural elucidation

Six compounds were isolated from *D. platyphylloide* and identification of six compounds whose structures are shown in **Figure 2**. The spectral properties of these known compounds, including ¹H and ¹³C-NMR data, were identical to those previously described in the literature.

15α-Acetoxyhopan-22-ol (1): white powder; HR-ESI-MS *m/z* 509.3980 [M+Na]⁺ (calcd. for $C_{32}H_{54}O_{-3}Na$, 509.3971) for $C_{32}H_{54}O_{3}$. ¹H and ¹³C NMR (500 and 125 MHz, CDCl₃) spectroscopy data, see **Table 2**. **Hopane-15α,22-diol (2)**: white powder; HR-ESI-MS *m/z* 467.3882 [M+Na]⁺ (calcd. for $C_{30}H_{52}O_{2}Na$, 467.3865) for $C_{30}H_{52}O_{2}$. ¹H and ¹³C NMR (400 and 100 MHz, CDCl₃) spectroscopy data, see **Table 2**.

Zeorin (3): white powder; HR-ESI-MS m/z 467.3880 [M+Na]⁺(calcd. for C₃₀H₅₂O₂Na, 467.3865) for C₃₀H₅₂O₂. ¹H and ¹³C NMR (400 and 100 MHz, DMSO-*d*₆) spectroscopy data, see **Table 2**.

Cerevisterol (4): white powder; HR-ESI-MS m/z453.3350 [M+Na]⁺(calcd. For C₂₈H₄₆O₃Na, 453.3345) for C₂₈H₄₆O₃. ¹H and ¹³C NMR (500 and 125 MHz, DMSO-*d*₆) spectroscopy data, see **Table 2**. **Salvigenin (5):** yellow powder; HR-ESI-MS m/z351.0839 [M+Na]⁺(calcd. for C₁₈H₁₆O₆Na, 351.0845) for C₁₈H₁₆O₆. ¹H and ¹³C NMR (500 MHz, DMSO-*d*₆) spectroscopy data, see **Table 3**.

5-Hydroxy-3',4',7-trimethoxyflavone (6): yellow powder; HR-ESI-MS m/z 351.0849 [M+Na]⁺ (calcd. for C₁₈H₁₆O₆Na, 351.0845) for C₁₈H₁₆O₆. ¹H and ¹³C NMR (500 MHz, DMSO- d_6) spectroscopy data, see **Table 3**.

Bioassays for Anticancer Activities

Six compounds isolated from *D. platyphylloides* (Nyl.) were tested the cytotoxic activity against four cancer cell lines: MCF-7, HeLa, NCI-H460, and HEP G2 by SRB assay method. Every sample was tested three times. The cytotoxic activity of these compounds expressed as a percentage of cell growth inhibition (I%) (**Table 1**). The results showed that (5) exhibited weak antiproliferative effect against Hep G2 cell lines and the others were inactive.

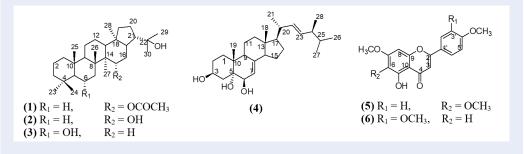


Figure 2: The chemical structures of isolated compoundsfrom D. platyphylloides (Nyl.).

Compound	Inhibition of cell g	Inhibition of cell growth (%)						
	MCF-7	HeLa	NCI-H460	HepG2				
1	15.2 ± 4.8	0.7 ± 8.5	6.2 ± 4.1	8.9 ± 2.4				
2	$39.7\pm\!5.0$	22.0 ± 1.3	29.8 ± 1.8	25.8 ± 4.7				
3	31.3 ± 3.0	9.3 ± 6.5	22.1 ± 2.1	3.4 ± 4.9				
4	59.4 ± 3.3	53.7 ± 3.3	29.5 ± 4.9	9.1 ± 4.1				
5	50.9 ± 1.6	24.8 ± 3.9	$29.2\pm\!\!2.2$	63.0 ± 2.2				
6	35.1 ± 1.6	43.3 ± 2.3	24.8 ± 1.1	33.1 ± 3.6				
Camptothecin ^c)	$45{,}4\pm2{,}8$	$48,1\pm0,7$	$80{,}8\pm2.0$	$57.0 \pm 1{,}6$				

Table 1: Inhibition of cytotoxic activity against four cancer cell lines of isolated compounds

 $^{a)}$ The compounds were tested at the concentration of 100 $\mu g/mL$

^{b)}The presented data are means of three experiments \pm SD.

^{c)}Camptothecin was tested as a positive control at the concentration of 0.01 μ g/mL for NCI-H460, 0.05 μ g/mL for MCF-7, 0.07 μ g/mL for HepG2, and of 1 μ g/mL for HeLa cells.

Pos.	(1)		(2)		(3)		(4)	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	-	40.3	-	40.6	-	40.9	-	32.4
2	-	16.2	-	18.9	-	18.0	-	31.2
3	-	41.9	-	42.1	-	43.5	3.76 m	65.9
4	-	33.3	-	33.3	-	33.3	1.86 <i>dd</i> (12.0,	39.9
							6.5)	
							1.50 d (5.0) -	
5	-	53.5	-	55.9	0.72 d (13.0)	59.9	-	74.4
6	-	22.5	-	19.1	3.74 ddd	66.5	3.37 d (5.0)	72.1
					(13.5, 9.5, 4.0)			
7	-	36.8	-	37.0	1.93 d (13.0)	44.7	5.08 dd (5.0, 3.0)	119.4
					1.37 d (3.6)			
8	-	43.4	-	43.7	-	42.1	-	139.6
9	-	50.7	-	50.6	-	49.3	-	42.2
10	-	37.4	-	37.7	-	38.5	-	36.6
11	-	18.7	-	21.0	-	21.3	-	21.3
12	-	24.1	-	24.3	-	23.6	-	38.9
13	-	50.1	-	49.1	-	48.9	-	43.0
14	-	46.5	-	47.3	-	41.4	-	54.1
15	5.07 <i>dd</i>	76.1	3.84 <i>dd</i>	75.0	-	33.9	-	22.6
	(11.0, 5.0)		(9.6, 5.2)					
16	-	25.9	-	32.8	-	20.6	-	27.7
17	-	52.9	-	50.7	-	53.8	-	55.3
18	-	44.2	-	44.4	-	43.5	0.54 <i>s</i>	12.0
19	-	41.6	-	41.1	-	40.9	0.91 s	17.7
20	-	26.6	-	27.0	-	26.0	2.02 <i>dd</i>	40.2
							(15.0, 6.5)	

Continued on next page

Table 2 con	ntinued							
Pos.	(1)		(2)		(3)		(4)	
21	2.21 dd	51.3	2.21 dd	50.6	2.10 dd	50.3	0.99 d (6.5)	20.9
	(20.0, 9.0)		(18.8, 11.2)		(20.0, 9.0)			
22	-	74.0	-	73.8	-	71.5	5.17 dd	135.4
							(15.0, 8.0)	
23	0.86 s	33.3	0.76 s	33.5	1.12 s	36.6	5.24 dd	131.4
							(15.0, 8.0)	
24	0.77 s	21.6	0.83 s	21.7	0.94 s	21.9	-	42.0
25	0.82 s	15.7	0.79 s	15.9	0.81 s	16.9	-	32.4
26	1.02 s	17.9	1.00 s	15.9	0.98 s	18.0	0.81 <i>d</i> (6.5)	19.4
27	1.08 s	12.6	1.05 s	11.9	0.92 s	16.8	0.80 <i>d</i> (6.5)	19.7
28	0.75 s	20.6	0.82 s	17.6	0.71 <i>s</i>	15.8	0.89 d (7.0)	17.2
29	1.16 s	29.0	1.17 s	28.9	1.03 s	28.9	-	-
30	1.19 s	31.0	1.20 s	31.1	1.07 s	30.7	-	
3-OH	-		-	-	-	-	4.22 d (5.5)	-
5-OH	-		-	-	-	-	3.58 s	-
6-OH	-	-	-	-	3.88 <i>d</i> (6.5)	-	4.49 d (5.5)	-
22-OH	-	-	-	-	3.81 s	-	-	-
16-	-	170.6	-	-	-	-	-	-
OCOCH	13							
15-CO-	-	22.1	-	-	-	-	-	-
15-	1.96 s		-	-	-	-	-	-
OCOCH	[3							

DISCUSSION

Compound (1) was isolated as a white powder. The molecular formula of C32H54O3 of (1) was determined from the ¹³C NMR data and an HRESIMS sodium adduct ion at m/z 509.3980 [M+Na]⁺(calcd. for C₃₂H₅₄O₃Na, 509.3971). The ¹H NMR data (Table 1) showed a methyl group at δ_H 1.96, an oxymethine signal at δ_H 5.07 (1H, dd, J_{aa} =11.0 Hz, J_{ae} =5.0, H-15 β), and methine proton at δ_H 2.21 (1H, dd, J = 20.0, 9.0 Hz, H-21). Its ¹³C NMR data showed 32 signals including two signals at δ_C 51.3 and 74.0 specialized for C-21 and C-22 of a 22-hydroxyhopane skeleton, a methyl carbon at δ_C 22.1 and a signal of carbonyl carbon at δ C 170.6. In the HMBC experiments, the correlations of H-15 to carbons at δ_C 46.5 (C-14), 25.9 (C-16), 52.9 (C-17), and 170.6 (C=O), of H-16 to carbons at δ_C 76.1 (C-15) and C-17, and of the methyl proton to carbonyl carbon supported the position of the acetyl group attached to C-15. Detailed analysis of the coupling constants of the signal proton H-15 indicated that the acetyl group at C-15 possessing the α -orientation. These spectroscopic data were compatible with the ones in the literature⁷. Thus, (1) was elucidated to be 15α -acetoxyhopan-22ol.

Compound (2) was isolated as a white powder. The ¹H NMR data of (2) (Table 2) was similar with that of (1) but it lacked an acetyl group at C-15. The oxymethine signal of proton at δ_H 5.07 in (1) was shifted to higher field compared to that of (2). The same observation was seen in the ¹³C NMR spectrum of (2) with the lack of an acetyl group and the replacement of the hydroxyl group at C-15. Detailed analysis of the coupling constants of H-15 at δ_H 3.84 (1H, dd, J_{ae} =9.6 Hz, and J_{aa} =5.2 Hz) suggesting the α orientation of the hydroxyl group at C-15. Furthermore, the molecular formula of (2) was determined as $C_{30}H_{52}O_2$ through the sodium adduct ion at m/z467.3882 [M+Na]⁺ in the HRESIMS spectrum. On the basis of above results, (2) was hopene- 15α , 22diol⁷.

Compound (3) was isolated as a white amorphous powder. The HR-ESI-MS spectrum of (3) showed a pseudomolecular ion peak at m/z 467.3880 [M+Na]⁺ (calcd. 467.3865), corresponding to the molecular formula of C₃₀H₅₂O₂. The ¹H NMR data (**Table 2**) revealed signals of eight methyl singlets at δ_H 0.71 (3H, s, H-28), 0.81 (3H, s, H-25), 0.92 (3H, s, H-27), 0.94 (3H, s, H-24), 0.98 (3H, s, H-26), 1.03 (3H, s, H-29), 1.07 (3H, s, H-30), and 1.12(3H, s, H-23), one methine proton at δ_H 2.10 (1H, *dd*, *J* = 20.0, 9.0 Hz, H-21). Detailed analysis of the coupling constants of the triplet of doublet proton signal at δ_H 3.74 (1H, *ddd*, J = 13.5, 9.5, 4.0 Hz, H-6 β) indicated that this proton was coupled to two axial protons at δ_H 0.72 (*d*, J_{aa} = 13.0 Hz, H-5 α) and 1.93 (*d*, J_{aa} = 13.0 Hz, H-7 α) and one equatorial proton at δ_H 1.37 (*d*, J_{ae} = 3.6 Hz, H-7 β). ¹³C NMR spectrum showed two signals at δ_C 50.3 and 71.5 specialize for C-21 and C-22 of 22-hydroxyhopane skeleton. The comparison of these spectroscopic data of (**3**) with those of zeorin in the literature⁸ showed good compatibility. Therefore, (**3**) was hopane-6 α ,22-diol or zeorin.

Compound (4) was isolated as a white amorphous powder. The HR-ESI-MS spectrum of (4) showed a pseudomolecular ion peak at m/z 453.3350 [M+Na]⁺ (calcd. 453.3345), corresponding to the molecular formula of C₂₈H₄₆O₃. The ¹H NMR spectrum recorded in DMSO- d_6 (Table 2) of (4) displayed signals for two tertiarymethyl groups with singlets at δ_H 0.54 (3H, H-18) and 0.91 (3H, H-19), four secondary methyl groups with doublets at δ_H 0.99 (3H, d, J= 6.5 Hz, H-21), 0.80 (3H, d, J= 6.5 Hz, H-27), 0.81 (3H, d, J = 6.5 Hz, H-26) and 0.89 (3H, d, J= 7.0 Hz, H-28), two oxymethines [δ_H 3.76 (1H, m, H-3 α) and 3.37 (1H, d, J= 5.0 Hz, H-6 α)], and three olefinic protons resonating at δ_H 5.08 (1H, dd, J= 5.0, 3.0 Hz, H-7), 5.17 (1H, *dd*, *J*= 15.0, 8.0 Hz, H-22), and 5.24 (1H, dd, J = 15.0, 8.0 Hz, H-23). The¹³C NMR (Table 2) spectrum showed the presence of 28 carbon signals including four olefinic carbon signals at δ_C 119.4 (C-7), 131.4 (C-23), 135.4 (C-22) and 139.6 (C-8), and three oxygenated carbons at δ_C 65.9 (C-3), 72.1 (C-6) and 74.4 (C-5). These data demonstrated that (4) was a cholestane derivative. The HMBC cross peaks of two oxymethine protons as well as a hydroxyl proton at δ_H 3.76, 3.37 and 3.58 with oxygenated carbons (C-3, C-6, and C-5, respectively) confirmed the position of three hydroxyl group at these carbons. Analysis of the spectral data of (4) and the comparison with cerevisterol in the literature⁹ suggested that (4) was cerevisterol.

Compound (5) was isolated as yellow powder and possessed a molecular formula of $C_{18}H_{16}O_6$, as defined by the ¹³C NMR and HRESIMS data at m/z 351.0839 [M+Na]⁺(calcd 351.0845). The ¹H NMR spectrum of (5) showed the *ortho* aromatic protons at δ_H 8.08 (2H, *d*, *J* =9.0 Hz, H-3', 5') and 7.13 (2H, *d*, *J* =9.0 Hz, H-2', 6'). Besides, the signals at δ_H 6.94 (1H, s, H-3), 6.97 (1H, s, H-8), 3.93 (3H, s, 7-OCH₃), 3.87 (3H, s, 4'-OCH₃), 3.74 (3H, s, 6-OCH₃) and a chelated hydroxyl at δ_H 12.88 (1H, s, 5-OH) proved (5) to be a flavone with characteristic for a 6,7,4'- trisubstituted flavone. This was further confirmed by the HMBC cross peaks of three methoxy

Table 3: ¹ H and ¹³ CNMR Spectroscopic Data for (5) and (6)							
Pos.	(5)		(6)				
	δ_H , (J in Hz)	δ_C	δ_H , (J in Hz)	δ_C			
2	-	163.6	-	163.6			
3	6.94 <i>s</i>	103.3	7.03 s	104.0			
4	-	182.2	-	182.0			
5	-	152.0	-	161.1			
5a	-	105.1	-	104.7			
6	-	131.9	6.38 <i>d</i> (1.5)	98.0			
7	-	158.7	-	165.2			
8	6.97 s	91.7	6.84 <i>d</i> (1.5)	92.8			
8a	-	152.7	-	157.3			
1'	-	122.7	-	122.7			
2'	7.13 <i>d</i> (9.0)	128.3	7.60 <i>d</i> (1.0)	109.5			
3'	8.08 d (9.0)	114.6	-	149.0			
4'	-	162.4	-	152.2			
5'	8.08 d (9.0)	114.6	7.14 <i>d</i> (8.5)	111.7			
6'	7.13 <i>d</i> (9.0)	128.3	7.72 <i>dd</i> (8.5; 1.0)	120.2			
5-OH	12.88 s	-	12.92 s	-			
6-OMe	3.74 s	60.0	-	-			
7–OMe	3.93 s	55.6	3.89 s	56.1			
3'-OMe	-	-	3.86 s	55.9			
4'-OMe	3.87 s	56.5	3.88 s	55.8			

able 3: 1 H and 13 CNMR Spectroscopic Data for (5) and (6

protons at δ_H 3.93, 3.87, and 3.74 with the aromatic carbon C-7 (δ_C 158.7), C-4' (δ_C 162.4), and C-6 (δ_C 131.9). Based on these spectral data as well as the comparison with the corresponding compound in the literature¹⁰, **5**) was assigned as 5-hydroxy-4',6,7trimethoxyflavone or salvigenin.

Compound (6) gave the similar number signals of ¹H and ¹³C NMR data to (5), except for the position of the substituted groups. The meta coupled of aromatic protons at δ_H 6.38 (1H, d, J = 1.5 Hz, H-6) and 6.83 (1H, d, J = 1.5 Hz, H-8), while the resonance at δ_H 7.72 (1H, dd, J = 8.5, 1.0 Hz, H-6') is ortho coupled to 7.14 (1H, d, J = 8.5 Hz, H-5') and meta coupled to 7.60 (1H, d, J = 1.0 Hz, H-2'). Moreover, the molecular formula of (6) was determined as C₁₈H₁₆O₆ through the sodium adduct ion at m/z 351.0849 [M+Na]⁺ (cald. 351.0845) in the HRESIMS spectrum. On the basis of above results, (6) was 5-hydroxy-3',4',7-trimethoxyflavone¹¹.

The isolated compounds from *Dendriscosticta platy-phylloides* (at the concentration of 100μ g/mL) were evaluated for their *in vitro* cytotoxic potential against 4 cancer cell lines MCF-7, HeLa, NCI-H460 and HepG2 by sulforhodamine B colorimetric assay method (SRB assay)¹¹ with the camptothecin as the positive control. Every sample was tested three times. In general, any tested compound with a percentage of inhibition higher than 50% may be potential anticarcinogen and was then be determined the IC₅₀ value. The results showed that (4) and (5) performed weak inhibitive activity on MCF 7 and HEP G2 cell lines with %I about 59.4 % and 63%, respectivity. The others showed no cytotoxic activity against four cell lines.

CONCLUSION

Six known compounds, 15α -acetoxyhopan-22-ol(1), hopane- 15α ,22-diol (2), zeorin (3), cerevisterol (4), salvigenin (5), and 5-hydroxy-3',4',7trimethoxyflavone (6) were isolated for the first time from the lichen *Dendriscosticta platyphylloides*. The cytotoxic activities of these compounds against HeLa, HepG2, NCI-H460 and MCF–7 cancer cell lines were inactive.

ABBREVIATIONS

¹H- NMR: Proton nuclear magnetic resonance
¹³C NMR: Carbon-13 nuclear magnetic resonance CDCl₃: Deuterochloroform *d*: doublet *dd*: doublet and double
DMSO: Dimethyl sulfoxide (CD₃SOCD₃)
EtOH: Ethanol (C₂H₅OH)
EtOAc: Ethyl acetate (CH₃COOCH₃)
HMBC: Heteronuclear multiple bond correlation
HRESIMS: High-resolution electrospray ionisation mass spectrometry
MeOH: Methanol (CH₃OH) *s*: singlet
TLC: Thin layer chromatography

COMPETING INTERESTS

The authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

Nguyen Thi My Dung, Huynh Thi Ngoc Tuyet and Do Thi My Lien have contributed in conducting experiments, getting hold of data and writing the manuscript. Ho Ky Quang Minh and Nguyen Tuan Hai were responsible for bioassay results. Nguyen Kim Phi Phung (corresponding author) and Jacques Mortier have contributed significantly explanation of data and revising the manuscript.

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