

DEPSIDONES WITH ALPHA-GLUCOSIDASE INHIBITION FROM THE LICHEN *USNEA CERATINA*

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ABSTRACT

From the lichen *Usnea ceratina*, eight γ -lactonic depsidones, 3'-demethylcryptostictinolide (**1**), 8'-hydroxycryptostictinolide (**2**), 8'-ethoxycryptostictinolide (**3**), vesuvianic acid (**4**), 8'-O-methylstictic acid (**5**), stictic acid (**6**), norstictic acid (**7**) and bailesidone (**8**) were identified by HR-ESI-MS, and NMR analyses. For the first time, compounds (**2**, **4-7**) were detected from the *Usnea* genus, whereas compounds **1** & **3** were previously reported from this species. Their α -glucosidase inhibitory properties were evaluated. All purified depsidones except **1** & **7** possessed better α -glucosidase inhibitory activity (IC_{50} values ranged from 38.05 to 143.94 μ M) than the standard drug acarbose (IC_{50} value of 214.50 μ M).

Keywords: *Usnea ceratina*, lichen, depsidone, γ -lactonic depsidone, α -glucosidase inhibitory activity.

1. Introduction

The *Usnea* genus comprising of more than 360 species belongs the Parmeliaceae family. It is one of mostly pale gray-green fruticose lichens (Prateeksha, 2016). Phytochemical investigations of *Usnea* species declared phenolics, depsides and depsidones were determined as the main compounds (Prateeksha, 2016). Further, benzofurans, terpenoids and steroids were notified (Prateeksha, 2016). In our earlier papers, depsidones (Bui, 2020), dibenzofuran and phenolic acid (Bui, 2021a) were purified from *U. ceratina*. As part of our favored studies on α -glucosidase inhibitors of Vietnamese plants (Nguyen, 2015; Nguyen, 2016a; Nguyen 2016b) as well as bioactive constituents of lichens (Huynh, 2020; Huynh, 2021a; Huynh, 2021b), this study disclosed the separation, structural identification, and α -glucosidase inhibitory property of

eight depsidones from *U. ceratina* collected from the bark trees at Paksong town, Paksong district, Champasack province, Laos.

2. Materials and methods

2.1 Materials

The thalli of the lichen *Usnea ceratina* Arch were collected at Paksong town, Paksong district, Champasack province, Laos in April 2015. The scientific name of the lichen was recognized by Dr. Harrie J. M. Sipman, Freie Universitaet, Berlin, Germany. A voucher specimen (US-B030) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, National University – Ho Chi Minh City, Vietnam.

2.2 Methods

2.2.1. General experimental procedures for isolation and structural identification

The HR-ESI-MS were recorded on an Exactive mass spectrometer (Thermo Fisher Scientific). The NMR spectra were measured on a Bruker Avance III spectrometer (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR). TLC was carried out on silica gel 60 F₂₅₄ or silica gel 60 RP-18 F₂₅₄S (Merck) and spots were visualized by spraying with a solution of 5% vanillin in ethanol, followed by heating at 100°C. Column chromatography was performed with silica gel 60 (0.040 – 0.063 mm, Merck).

2.2.2. Extraction and isolation

The fresh lichen thalli (1.60 kg) were cleaned under running tap water and air-dried. The ground powder (1.15 kg) was extracted with acetone at room temperature. After filtration, the solvent was removed under reduced pressure to yield the crude acetone residue (163.0 g). This residue was then subjected to silica gel solid phase extraction and further eluted with chloroform, ethyl acetate to deliver chloroform extract (60.0 g) and ethyl acetate (12.0 g) extract.

The chloroform extract (60.0 g) was fractionated using silica gel column chromatography (CC) with the solvent systems of *n*-hexane–chloroform to afford nine sub-fractions (C1–C9). The sub-fraction C5 (5.05 g) was rechromatographed on silica gel CC with solvents *n*-hexane–chloroform (8:2, v/v) to give **5** (6.0 mg), **6** (5.0 mg) and **8** (6.0 mg). The sub-fraction C6 (4.83 g) was applied to silica gel CC using eluted solvents *n*-hexane–chloroform (7:3) to get **4** (6.5 mg).

The ethyl acetate extract (12.0 g) was separated on silica gel CC using chloroform–methanol mixture with increasing methanol to yield seven sub-fractions (EA1– EA7). The sub-fraction EA4 (1.5 g) was rechromatographed eluting with chloroform–methanol (98:2, v/v) to deliver **1** (5.0 mg), and **3** (6.5 mg). The sub-fraction EA5 (2.05 g) was purified on silica gel chromatographic column using chloroform–methanol–acetic acid (95:5:1, v/v/v) to give **2** (7.0 mg). The sub-fraction EA7 (1.8 g) was applied to silica gel CC, eluted with chloroform–methanol (9:1, v/v) to afford **1** (4.5 mg) and **7** (5.0 mg).

3'-Demethylcryptostictinolide (**1**): HR-ESI-MS m/z 357.0577 [M-H]⁻ (calcd for C₁₈H₁₃O₈, 357.0611); ^1H & ^{13}C -NMR data (DMSO-*d*₆) see Table 1.

8'-Hydroxycryptostictinolide (**2**): HR-ESI-MS m/z 389.0882 [M+H]⁺ (calcd for C₁₉H₁₇O₉, 389.0873); ^1H & ^{13}C -NMR data (DMSO-*d*₆) see Table 1.

8'-Ethoxycryptostictinolide (**3**): HR-ESI-MS m/z 415.0987 [M-H]⁻ (calcd for C₂₁H₁₉O₉, 415.1029); ^1H & ^{13}C -NMR data (DMSO-*d*₆) see Table 1.

Vesuvianic acid (**4**): HR-ESI-MS m/z 437.0826 [M+Na]⁺ (calcd for C₂₁H₁₈O₉Na, 437.0849); ^1H & ^{13}C -NMR data (DMSO-*d*₆) see Table 1.

8'-*O*-Methylstictic acid (**5**): HR-ESI-MS m/z 423.0651 [M+Na]⁺ (calcd for C₂₀H₁₆O₉Na, 423.0692). ^1H & ^{13}C -NMR data (DMSO-*d*₆) see Table 1.

Stictic acid (**6**): HR-ESI-MS m/z 385.0557 [M-H]⁻ (calcd for C₁₉H₁₃O₉, 385.0560); ^1H & ^{13}C -NMR data (DMSO-*d*₆) see Table 1.

Norstictic acid (**7**): HR-ESI-MS m/z 371.0461 $[M-H]^-$ (calcd for $C_{18}H_{11}O_9$, 371.0403); 1H & ^{13}C -NMR data (DMSO- d_6) see Table 1.

Bailesidone (**8**): HR-ESI-MS m/z 413.0854 $[M-H]^-$ (calcd for $C_{21}H_{17}O_9$, 413.0854); 1H & ^{13}C -NMR data (acetone- d_6) see Table 1.

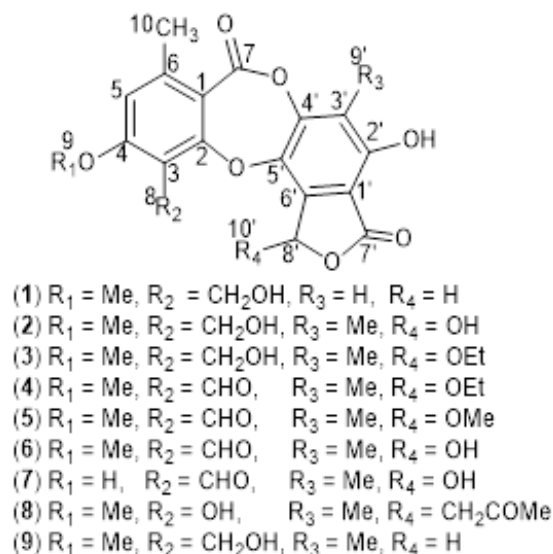


Figure 1: Chemical structures of compounds 1-9.

2.2.3. α -Glucosidase inhibitory activity assay

The α -glucosidase inhibitory activity was determined according to those presented in our previous paper (Nguyen, 2015; Nguyen, 2016a; Nguyen 2016b). 25 μL of *p*-nitrophenyl- α -D-glucopyranoside (3 mM), 25 μL of α -glucosidase enzyme 0.2 U/mL in 0.01 M phosphate buffer solution (pH = 7) were added to 625 μL of the sample solution (compounds **1-8**). Each reaction was carried out at 37°C for 30 minutes, and stopped by adding 375 μL of Na_2CO_3 (0.1 M),

measured the optical density at 401 nm. The IC_{50} values were calculated as the concentration of α -glucosidase inhibitor that inhibited 50% of α -glucosidase activity. Acarbose was used as the positive control.

3. Results and discussion

Compound **1** was afforded as a white amorphous powder. The molecular formula was confirmed as $C_{18}H_{14}O_8$ by HR-ESI-MS data ($[M-H]^-$ m/z 357.0577, calcd. 357.0611). The 1H -NMR data of **1** (Table 1) showed two hydroxyl protons at δ_H 11.20 (1H, *s*, OH-2'), 5.02 (1H, *s*, OH-8), two aromatic protons at δ_H 6.96 (1H, *s*, H-5), 6.37 (1H, *s*, H-3'), two oxymethylene groups at δ_H 4.58 (2H, *s*, H-8), 5.64 (2H, *s*, H-8'), one oxymethyl group at δ_H 3.87 (3H, *s*, H-9), and three methyl protons at δ_H 2.43 (3H, *s*, H-10). The ^{13}C -NMR data of **1** (Table 1) displayed eighteen carbons, including two carbonyl carbons at 167.2 (C-7), 172.0 (C-7'), one methyl carbon at δ_C 21.0 (C-10), one methoxy carbon at δ_C 56.3 (C-9), two oxymethylene carbons at δ_C 51.0 (C-8), 66.1 (C-8'), and twelve aromatic carbons with five of those were oxygenated, two of them were methine aromatic carbons. On other hands, protons at δ_H 5.64 (H-8') correlated with carbons at δ_C 107.7 (C-1'), 140.9 (C-6'), and 172.0 (C-7') in HMBC, were signified γ -lactone ring. Those data of **1** were suggested a depsidone with γ -lactone moiety similar cryptostictinolide (**9**) lacked one methyl carbon at C-3'. The HMBC spectrum of **1** (Figure 2) exhibited correlations between proton at δ_H 6.96 (H-5) and

carbon at δ_C 21.0 (C-10), protons at δ_H 4.58 (H-8), 3.87 (H-9) and carbon at δ_C 161.5 (C-4), proton at δ_H 6.37 (H-3') and carbon at δ_C 154.9 (C-2'), were indicated the arrangement of these substituents in the depsidone framework. Hence, the structure of **1** identified as **3'-demethylcryptostictinolide** (Dévéhat, 2007) was determined for the first time from this species.

Compound **2** was given as a white amorphous powder. The molecular formula was affirmed as $C_{19}H_{16}O_9$ ($[M+H]^+$ m/z 389.0882, calcd. 389.0873). The ^{13}C & 1H -NMR spectra of **2** were similar to those of **9**, but missed one oxymethylene carbon C-8', and revealed one acetal carbon at δ_C 95.4 (C-8')/ δ_H 6.96 (1H, *d*, 7.5, H-8') in **2**, further the upfield shift of carbon C-7' at δ_C 166.5, which were evenced that a hydroxyl group was attached to be C-8' of a depsidone skeleton. Thence, the structure of **2** was elucidated as **8'-hydroxycryptostictinolide** (Ismed, 2017).

Compound **3** was yielded as a white amorphous powder. The molecular formula was evinced as $C_{21}H_{20}O_9$ ($[M-H]^-$ m/z 415.0987, calcd. 415.1029). The ^{13}C & 1H -NMR spectra of **3** exposed signals of a depsidone frame were similar to that of **2**, except for the presence of an ethoxy function at δ_C 64.6 (C-10')/3.85 (2H, *m*, H-10'), 15.1 (C-11')/1.25 (3H, *t*, 7.0, H-11'), and further downfield shift of carbon C-8' at δ_C 99.3 in **3**. The HMBC spectrum of **3** (Figure 2) proved correlations between protons at δ_H 3.85 (H-10') and

carbon C-8', were evenced that an ethoxy group was linked to be C-8' of skeleton. Therefore, the structure of **3** verified as **8'-ethoxycryptostictinolide** (Bui, 2021b) was elucidated for the first time from this species.

Compound **4** was delivered as a white amorphous powder. The molecular formula was illustrated as $C_{21}H_{18}O_9$ by HR-ESI-MS data ($[M+Na]^+$ m/z 437.0826, calcd. 437.0849). The NMR spectral data of **4** (Table 1) were similar to those of **3**, except for the arriving of one formyl function at C-3 at δ_C 186.9 (C-8)/ δ_H 10.53 (1H, *s*, H-8) in **4**, instead of carbinol group at δ_C 51.1 (C-8) in **3**. Furthermore, this proton at δ_H 10.53 (H-10) correlated with carbon at δ_C 115.0 (C-3) in HMBC (Figure 2), and besides upfield shift of this carbon were assigned that a formyl moiety was connected to be C-3 of depsidone. Thus, the structure of **4** was testified as **vesuvianic acid** (Huneck, 1987).

Compound **5** was yielded as a white amorphous powder. The molecular formula was evidenced as $C_{20}H_{16}O_9$ by HR-ESI-MS data ($[M+Na]^+$ m/z 423.0651, calcd. 423.0692). The ^{13}C & 1H -NMR data of **5** (Table 1) were similar to those of **4**, except for the disappearing of one ethoxy group in **4**, instead of methoxy moiety at δ_C 57.5 (C-10')/ δ_H 3.63 (3H, *s*, H-10') in **5**. Moreover, the correlation between protons at δ_H 3.63 (H-10') and carbon at δ_C 102.9 (C-8') observed in HMBC (Figure 2) was verified that a methoxy group was linked to be C-8' of depsidone. Consequently, the structure

of **5** testified as 8'-*O*-methylstictic acid (Shimada, 1980) was reported form this species (Bui, 2020).

Compound **6** was got as a white amorphous powder. The molecular formula was determined as $C_{19}H_{14}O_9$ by HR-ESI-MS data ($[M-H]^-$ m/z 385.0557, calcd. 385.0560). The ^{13}C & 1H -NMR data of **7** (Table 1) were similar to those of **2**, except disappeared carbinol function in **2**, and replaced formyl group at C-3 at δ_C 186.8 (C-8)/ δ_H 10.46 (1H, *s*, H-8) in **6**. Additionally, the correlation between this proton at δ_H 10.46 (H-8) and carbon at δ_C 114.5 (C-3) in HMBC (Figure 2) was clarified that hydroxyl was substituted to be C-3. Accordingly, the structure of **6** designated as **stictic acid** (Shimada, 1980) was isolated form this species (Bui, 2020).

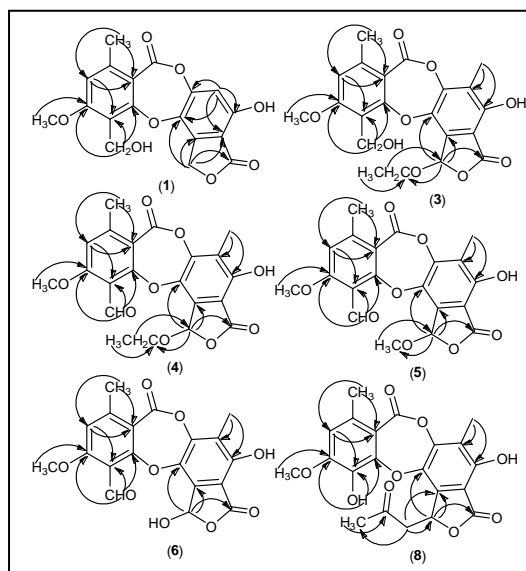


Figure 2: Key HMBC correlations of compounds **1**, **3-6**, **8**.

Compound **7** was afforded as a white amorphous powder. The

molecular formula was proved as $C_{18}H_{12}O_9$ by HR-ESI-MS data ($[M-H]^-$ m/z 371.0461, calcd. 371.0403). The ^{13}C & 1H -NMR data of **7** (Table 1) were similar to those of **6**, but missed methoxy carbon at C-4 at δ_C 56.9 (C-9)/ δ_H 3.91 (1H, *s*, H-9) in **6**, and downfield shift of carbon C-4 at δ_C 164.2 in **7**, were testified a hydroxyl was attached to be C-4. Thus, the structure of **7** was determined as **norstictic acid** (Shimada, 1980).

Compound **8** was gave as a white amorphous powder. The molecular formula was proved as $C_{21}H_{18}O_9$ by HR-ESI-MS data ($[M-H]^-$ m/z 413.0854, calcd. 413.0854). The ^{13}C & 1H -NMR data of **8** (Table 1) were similar to those of **1**, but lacked oxymethylene carbon at C-3 at δ_C 51.0 (C-8)/ δ_H 4.58 (2H, *s*, H-8) in **1**, and downfield shift of carbon C-3 at δ_C 133.3 in **8**, were testified a hydroxyl was attached to be C-3. Moreover, ^{13}C & 1H -NMR data of **8** were further possessed an acetomethyl moiety, following one carbonyl, one methylene, and one methyl carbons at δ_C 202.6 (C-11'), 46.6 (C-10'), 9.0 (C-12'), respectively, which were correlated with protons at δ_H 3.09 (1H, *dd*, 17.5 & 9.0, H-10'a), 3.71 (1H, *dd*, 17.5 & 2.5, H-10'b), and 2.23 (3H, *s*, H-12'), respectively. Additionally, protons at δ_H 3.09 (H-10'a), 3.71 (H-10'b) correlated with carbon at δ_C 77.2 (C-8') in HMBC (Figure 2), were identified that an acetomethyl function was linked to be C-8'. Thus, the structure of **8** signed as **bailesidone** was identified form this species (Bui, 2020).

Table 1: ^1H and ^{13}C -NMR data of 1-8 in $\text{DMSO}-d_6$

No.	^{13}C -NMR (δ ppm)								^1H -NMR (δ ppm, J in Hz)							
	1	2	3	4	5	6	7	8 ^a	1	2	3	4	5	6	7	8 ^a
1	112.8	111.6	112.6	113.2	115.0	113.2	111.9	114.9								
2	158.8	158.9	158.8	162.6	161.5	163.2	166.5	152.7								
3	118.0	118.5	118.2	114.2	115.9	114.5	110.2	133.3								
4	161.5	161.7	161.6	162.8	164.4	162.6	164.2	152.8								
5	111.6	112.7	111.7	112.9	113.5	112.9	117.5	112.2	6.96 s	6.95 s	7.00 s	7.13 s	7.11 s	7.08 s	6.88 s	6.87 s
6	144.2	144.2	144.5	151.3	151.9	151.0	152.0	134.3								
7	167.2	160.0	161.3	160.6	158.9	160.8	160.8	162.0			4.80 <i>dd</i> , 11.0 & 5.0					
8	51.0	51.3	51.1	186.6	187.3	186.8	190.8		4.58 s	4.61 s		10.47 s	10.52 s	10.46 s	10.46 s	
9	56.3	56.2	56.3	56.9	57.2	56.9										
10	21.0	20.9	20.9	21.6	21.9	21.6	21.6	56.8	3.87 s	3.87 s	3.90 s	3.95 s	4.01 s	3.91 s		3.92 s
1'	108.4	109.0	108.8	108.8	109.0	109.3	109.3	108.7	2.43 s	2.44 s	2.45 s	2.51 s	2.54 s	2.49 s		2.39 s
2'	154.9	151.5	151.9	152.4	151.9	152.0	151.9	150.3								
3'	107.8	120.4	121.2	121.6	121.9	120.9	120.9	119.0	6.37 s							
4'	149.1	148.3	148.5	148.3	150.2	148.1	148.1	149.1								
5'	135.5	137.9	137.9	137.4	138.6	137.6	137.6	138.0								
6'	140.9	135.9	133.3	133.5	133.8	136.0	136.0	137.0								
7'	172.0	166.5	166.0	166.0	168.3	166.5	163.6	170.5								
8'	66.1	95.4	99.3	94.4	102.9	95.2	95.2	77.2	5.64 s	6.96 <i>d</i> , 7.5	6.95 s	6.57 s	6.53 s	6.60 s	6.08 <i>m</i>	
9'	9.5	9.5	9.7	9.7	9.4	9.7	9.7	9.0	2.10 s	2.19 s	2.19 s	2.21 s	2.26 s	2.19 s	2.19 s	3.09 <i>dd</i> , 9.0, 17.5
10'			64.6	64.6	57.5		46.6			3.85 <i>m</i>	3.73 <i>m</i>		3.63 s			2.22 s
11'			15.1	14.7			202.6			1.25 <i>t</i> , 7.0	1.13 <i>t</i> , 7.0					3.71 <i>dd</i> , 2.5, 17.5
12'							9.0									2.23 s
8-OH									5.02 s	4.83 s	5.00 <i>dd</i> ,					
2'-OH									11.20 s	10.24 s	5.5 & 5.5					
8'-OH										8.22 s	10.24 s			8.21 s	8.21 s	

^aAcetone- d_6

Table 2: α -Glucosidase inhibition of compounds 1-8

Samples	Inhibition (%)				IC ₅₀ (μ M)
	100	50	25	10	
1	7.30 \pm 1.41	1.01 \pm 0.59	–	–	>250
2	79.52 \pm 1.14	24.95 \pm 1.93	9.62 \pm 1.70	2.17 \pm 0.70	73.13
3	46.42 \pm 0.30	35.40 \pm 0.61	23.84 \pm 0.41	–	126.62
4	99.41 \pm 0.57	68.52 \pm 1.53	30.01 \pm 0.75	7.47 \pm 0.63	38.05
5	46.79 \pm 0.75	19.35 \pm 0.69	2.43 \pm 1.02	–	110.11
6	37.05 \pm 0.76	21.12 \pm 1.10	6.61 \pm 1.03	–	143.94
7	17.72 \pm 2.13	6.94 \pm 1.12	1.35 \pm 0.97	–	>250
8	53.02 \pm 0.42	29.61 \pm 0.32	18.44 \pm 0.21	14.81 \pm 0.33	97.12
Acarbose					214.50

- Inhibition < 1%

All separated depsidones except **1** & **7** expressed potential inhibition against α -glucosidase enzyme (IC₅₀ values ranged from 38.05 to 143.94 μ M). Among them, **4** displayed the strongest effect on α -glucosidase inhibition (IC₅₀ value of 38.05 μ M) comparing with the acarbose drug (IC₅₀ value of 214.50 μ M).

4. Conclusion

Eight depsidones with γ -lactone moiety, including 3'-demethylcryptostictinolide (**1**), 8'-hydroxycryptostictinolide (**2**), 8'-ethoxycryptostictinolide (**3**), vesuvianic acid (**4**), 8'-O-methylstictic

acid (**5**), stictic acid (**6**), norstictic acid (**7**), and baileisidone (**8**) were elucidated from the lichen *Usnea ceratina* using HR-ESI-MS, and NMR spectroscopic data. All γ -lactone-type depsidones except **1** & **3** were informed from the genus *Usnea*, while, compounds **1** & **3** was announced from this species for the first time. All isolated depsidones except **1** & **7** exhibited better α -glucosidase inhibition (IC₅₀ values ranged from 38.05 to 143.94 μ M) than the acarbose drug (IC₅₀ value of 214.50 μ M).

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DEPSIDONE VỚI HOẠT TÍNH ỨC CHẾ ALPHA-GLUCOSIDASE TỪ ĐỊA Y *USNEA CERATINA*

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ABSTRACT

Từ địa y *Usnea ceratina*, tám hợp chất γ -lactonic depsidone đã được cô lập và định danh bằng các phương pháp hóa lý hiện đại kết hợp với khối phổ phân giải cao bao gồm 3'-demethylcryptostictinolide (**1**), 8'-hydroxycryptostictinolide (**2**), 8'-ethoxycryptostictinolide (**3**), vesuvianic acid (**4**), 8'-O-methylstictic acid (**5**), stictic acid (**6**), norstictic acid (**7**) và baileisidone (**8**). Đây là lần đầu tiên các hợp chất (**2**, **4-7**) được cô lập trong chi *Usnea*. Tất cả các hợp chất cô lập được xác định hoạt tính ức chế α -glucosidase, kết quả cho thấy depsidone **1** & **7** có khả năng ức chế tốt hơn (giá trị IC_{50} dao động từ 38.05 đến 143.94 μ M) thấp hơn nhiều so với chất chứng dương acarbose (giá trị IC_{50} 214.50 μ M).

Từ khóa: *Usnea ceratina*, lichen, depsidone, γ -lactonic depsidone, hoạt tính ức chế α -glucosidase