Research Article

Pham Van Khang*, Hoang Phu Hiep, Tu Quang Trung

Two new compounds from leaves of *Capparis dongvanensis* (Sy, B. H. Quang & D. V. Hai) and inhibition activities

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Abstract: In continuation of the research, the phytochemical investigation of *Capparis dongvanensis* (Sy, B. H. Quang & D. V. Hai) (Capparaceae). In this work, we described the isolation and identification of two novel polycyclic compounds Capparisone A (**1**) and Capparisone B (**2**). The structures of these compounds were determined based on NMR spectral data and mass spectrometry. Furthermore, we evaluated the inhibitory activities of isolated compounds from *C. dongvanensis* leaves against five cancer cell lines, the results showed that isolated compounds exhibited good activities on four cancer cell lines. Otherwise, two isolated compounds **1** and **2** and EA extract also exhibited the antioxidant activity in the DPPH free radical scavenging assay.

Keywords: *Capparis dongvanensis*, VietNam, anti-cancer activity, Lao Cai, *Capparis*

1 Introduction

Capparis genus in the Capparaceae family is mainly distributed in tropical, subtropical, and temperate regions [1]. In Vietnam, *Capparis* genus was found in the northern mountain region [2]. *Capparis* plants are used as an herbal medicine for the treatment of cough and other respiratory diseases [3].

In previous works, natural compounds had been isolated from some species of *Capparis* such as alkaloids, flavonoids, fatty acids, glucosinolates, isothiocyanates, amino acids, sterols, and other compounds [3]. Alkaloids were found in *Capparis spinosa* [4], *Capparis aegyptia*, *Capparis deserti*, and *Capparis leucophylla* [5], and *Capparis sepiaria* [6]. From *C. spinosa* plant, some alkaloids have been isolated, including capparispine, capparispine 26-*O*-*β*-D-glucoside, cadabicine 26-*O*-*β*-D-glucoside hydrochloride [7–9], capparisine A, capparisine B, and capparisine C [7,8]. Flavonoids in *Capparis* genus were identified in *Capparis corymbosa* Lam. roots [9] and *C. spinosa* buds with total flavonoids of about 1.13% [10], and flavones were isolated in leaves of *C. spinosa* [11,12] in ethanol extract. Derivatives of quercetin and kaempferol were isolated from methanolic extract of *C. spinosa* (Bonina et al. [13]) and *Capparis humilis* [14]. Isoginkgetin [12], isoquercitin [15], and isorhamnetin [8] are some biflavonoids that were isolated from the fruit of *C. spinosa*. Sterols are one of the rich compositions in species of the *Capparis* genus [3]. In *Capparis orientalis* and *Capparis sicula* ssp. *sicula*, sterols have 1–2% (w/w) of non-polar compositions [3], *C. spinosa* seed oil has 2,240.4 mg/kg of total non-polar compositions [3], *Capparis ovata* has 4875.5–12189.1 mg/kg, and *C. spinosa* has 4961.8–10009.1 mg/kg of total non-polar compositions [16]. To date, more than 15 sterols were isolated and identified in *C. ovata* [16]. *Capparis formosana* stems [17] ether extract, *C. spinosa* [15] fruits, *C. spinosa* [18], *C. sepiaria* leaves [19], and *Capparis decidua* root bark [20]. Otherwise, from *Capparis* genus also isolated triterpenoids and volatile compounds, glucosinolates, phenolic acid, and isothiocyanates [3,9,21].

The biological activities of *Capparis* genus exhibited a wide spectrum of activities, such as anti-cancer, antioxidant, anti-inflammatory and immunological, antirheumatic, antidiabetic, and anti-infective activities [3,9,13,22]; among them, the anticancer effect is like the most important activities. Methanolic extract of *C. decidua* inhibited ovarian (1A9), lung (A549), ileocecal (HCT-8), breast (MCF-7), nasopharyngeal (KB), and vincristine-resistant (KB-VIN) human tumor cell lines with ED50 ≤4 μg/mL (mean GI_{50} 15.1 μM) [3,9]. Extract from seeds of *C. spinosa* inhibited the proliferation of HepG2 cells, colon cancer HT29

* **Corresponding author: Pham Van Khang,** Faculty of Chemistry, Thai Nguyen University of Education, 20 Luong Ngoc Quyen Street, Thai Nguyen City 24000, Vietnam, e-mail: khangpv@tnue.edu.vn, tel: +84 917 146 083
Hoang Phu Hiep, Tu Quang Trung: Faculty of Biology, Thai Nguyen University of Education, 20 Luong Ngoc Quyen Street, Thai Nguyen City 24000, Vietnam

cells, and breast cancer MCF-7 cells with IC_{50} at about 1, 40, and 60 mM, respectively [3,9].

With diverse chemical compositions of genus *Capparis* with many groups of compounds, such as alkaloids, flavonoids, sterols, and aromatic acids [3,9], and a range of good biological activities, especially inhibitory activity against cancer cell lines [3,9], will be a basic foundation for research on other species of genus *Capparis*. In our previous work, we isolated 14 compounds from leaves of *Capparis dongvanensis* and evaluated the inhibitory activity of ethanolic extracts [23]. In this work, we continue to study the chemical compositions of *C. dongvanensis* stem and evaluate the inhibition activities against cancer cell lines.

2 Materials and methods

2.1 Plant materials

The sample plant used in this work was collected in Lao Cai, in May 2020, a mountain province in the northeast region of Vietnam, and authenticated by Dr. S.D.Thuong at Thai Nguyen University of Education, Vietnam, with a voucher specimen TNUE202005. After collection, the sample was dried at 50°C for 5 h.

2.2 Isolation of compounds

The air-dried leaves (3.5 kg) of *C. dongvanensis* were collected in Lao Cai, VietNam, followed by extraction in 90% aqueous ethanol as a solvent three times at 70°C. The ethanol solvent was removed under vacuum to afford a crude extract (210 g). A part of this crude extract (105 g) was subsequently extracted with dichloromethane (DCM), ethyl acetate (EA), and *n*-BuOH (Bu), respectively. The excessive solvent was removed under vacuum to afford crude extracts: DCM (13 g), EA (32 g), and Bu (45 g).

Crude EA extract (30 g) was chromatographed with silica gel using acetone/hexane gradient (1/20–1/1, v/v) to obtain six fractions (F1–F6). Fraction F2 was obtained through silica gel purification using acetone/hexane (1/10–1/1, v/v) as the mobile phase to give four sub-fractions. From sub-fraction 2.2, compound **1** (15 mg) was obtained using silica gel column chromatography with DCM/hexane (1/5–1/1, v/v). Sub-fraction 2.4 was subjected to preparative chromatography with acetone/hexane (1/5–1/2, v/v) to afford compound **2** (18 mg).

Compound **1** as colorless amorphous has a molecular ion peak at *m/z* 357.1461 $[M+Na]^+$ (100%) on the high resolution mass spectrum; ^1H NMR (ppm in $CDCl_3$) data of compound **1** (Table 1): 5.03 (d, *J* = 10.3 Hz, 1H, H-3), 3.38 (m, 1H, H-4), 6.03 (dd, *J* = 6.7, 1.1 Hz, 1H, H-5), 6.82 (d, *J* = 6.7 Hz, 1H, H-6), 1.31 (d, *J* = 7.0 Hz, 2H, H-7), 1.80 (d, *J* = 7.0 Hz, 2H, H-8), 6.29 (dd, *J* = 6.5, 1.0 Hz, 1H, H-9), 6.90 (d, *J* = 6.5 Hz, 1H, H-10), 6.83 (d, *J* = 6.5 Hz, 1H, H-1′), 6.69 (d, *J* = 6.5 Hz, 1H, H-2′), 6.72 (d, *J* = 1.5 Hz, 1H, H-5′), 3.81 (s, 3H, H-1″), 3.82 (s, 3H, H-2″), 1.20 (d, *J* = 6.8 Hz, 3H, H-3″); ^{13}C NMR (ppm in $CDCl_3$, 135 MHz) data of compound **1**: 146.59 (C-2), 93.79 (C-3), 45.63 (C-4), 129.48 (C-4a), 123.49 (C-5), 119.97 (C-6), 133.28 (C-6a), 17.59 (C-7), 18.37 (C-8), 130.94 (C-9), 108.94 (C-10), 132.22 (C-10a), 146.68 (C-11), 114.08 (C-1′), 113.33 (C-2′), 145.80 (C-3′), 144.16 (C-4′), 109.29 (C-5′), 132.11 (C-6′), 56.00 (C-1″), 55.96 (C-2″), and 29.71 (C-3″).

Compound **2** has a colorless amorphous and has a molecular ion peak in its mass spectrometry (MS) high-resolution spectrum at *m/z* 369.0957 $[M+Na]^+$ (90%): ^1H NMR (ppm in $CDCl_3$) data of compound **2** (Table 2): 6.67 (d, *J* = 7.9 Hz, 1H, H-3), 6.20 (s, 1H, H-4), 2.52 (td, *J* = 7.4, 5.6 Hz, 1H, H-9), 5.59 (m, 1H, H-10), 2.17 (dd, *J* = 14.7, 7.4 Hz, 1H, H-13a), 2.00 (dd, *J* = 14.7, 7.4 Hz, 1H, H-13b), 2.61 (d, *J* = 5.6 Hz, 1H, H-14), 3.62 (d, *J* = 3.2 Hz, 1H, H-1′), 0.99 (d, *J* = 7.2 Hz, 3H, H-2′), 3.62 (s, 3H, H-3′), 5.88 (s, 2H,

Table 1: The chemical shift of Capparisone A (1) (500 MHz in $CDCl_3$)

Entry	^{13}C (ppm)	^1H (*J* Hz, *δ* ppm)
2	146.59	
3	93.79	5.03 (d, *J* = 10.3 Hz, 1H)
4	45.63	3.38 (m, 1H)
4a	129.48	
5	123.49	6.03 (dd, *J* = 6.7, 1.1 Hz, 1H)
6	119.97	6.82 (d, *J* = 6.7 Hz, 1H)
6a	133.28	
7	17.59	1.31 (d, *J* = 7.0 Hz, 2H)
8	18.37	1.80 (d, *J* = 7.0 Hz, 2H)
9	130.94	6.29 (dd, *J* = 6.5, 1.0 Hz, 1H)
10	108.94	6.90 (d, *J* = 6.5 Hz, 1H)
10a	132.22	
11	146.68	
1′	114.08	6.83 (d, *J* = 6.5 Hz, 1H)
2′	113.33	6.69 (d, *J* = 6.5 Hz, 1H)
3′	145.80	
4′	144.16	
5′	109.29	6.72 (d, *J* = 1.5 Hz, 1H)
6′	132.11	
1″	56.00	3.81 (s, 3H)
2″	55.96	3.82 (s, 3H)
3″	29.71	1.20 (d, *J* = 6.8 Hz, 3H)

Table 2: The chemical shift of Capparisone B (2) (500 MHz in $CDCl_3$)

Entry	^{13}C (ppm)	^{1}H (*J* Hz, *δ* ppm)
1	202.02	
2	118.17	
3	107.31	6.67 (d, *J* = 7.9 Hz, 1H)
4	122.01	6.20 (s, 1H)
5	152.13	
6	147.06	
7	133.11	
8	57.16	
9	37.89	2.52 (td, *J* = 7.4, 5.6 Hz, 1H)
10	132.16	5.59 (m, 1H)
11	145.93	
12	190.18	
13	32.82	2.17 (dd, *J* = 14.7, 7.4 Hz, 1H, Ha), 2.00 (dd, *J* = 14.7, 7.4 Hz, 1H, Hb)
14	55.22	2.61 (d, *J* = 5.6 Hz, 1H)
1′	66.51	3.62 (d, *J* = 3.2 Hz, 1H)
2′	16.94	0.99 (d, *J* = 7.2 Hz, 3H)
3′	54.60	3.62 (s, 3H)
4′	100.22	5.88 (s, 2H)

H-4′); ^{13}C NMR (ppm in $CDCl_3$, 135 MHz) data of compound **2**: 202.02 (C-1), 118.17 (C-2), 107.31 (C-3), 122.01 (C-4), 152.13 (C-5), 147.06 (C-6), 133.11 (C-7), 133.11 (C-7), 37.89 (C-9), 132.16 (C-10), 145.93 (C-11), 190.18 (C-12), 32.82 (C-13), 55.22 (C-14), 66.51 (C-1′), 16.94 (C-2′), 54.60 (C-3′), 100.22 (C-4′).

2.3 Proliferation assay

The anti-proliferative property of compounds **1** and **2** was evaluated using the MTT assay [13,24]. The values of IC_{50} ± SEM were calculated by using the Logit method. In this work, ellipticine was used as a control.

2.4 DPPH free radical assay

The antioxidant activities of samples were experimented by using the DPPH free radical scavenging assay [22]. Fisrtly, add the 100 µL of each type of samples at concentrations of 1; 40; 80; 160; 320; 640; 1,280 µg/mL into test tubes, then add 2.9 mL of 0.1 mM DPPH disolved in methanol into test tubes, shake well and allow to stand for 30 minutes and then measured the absorbance on UV-VIS 1700 Shimadzu system at wavelength of 517 nm. The positive antioxidant control as quercetin was prepared similarly above. The results were presented as a percentage of DPPH inhibition (%) according to the following equation: DPPH% = (Abs control − Abs sample) × 100/Abs control.

The value of EC_{50} is the concentration of sample with DPPH free radical scavenging capacity of 50% [22].

3 Results and discussion

3.1 Structure elucidation of the new compounds

Compound **1**, a colorless amorphous, has a molecular ion peak *m/z* 357.1461 $[M+Na]^+$ (100%) on the high-resolution mass spectrum. The result of elemental analysis of compound **1** is as follows: C 79.023%, H 6.631%, and O 14.352%. In combination with NMR spectral data, compound **1** has the molecular formula as $C_{22}H_{22}O_3$ (cal. $C_{22}H_{22}O_3Na$, *m/z* 357.1466).

The ^1H NMR spectrum of compound **1** gives a methyl group signal at δ_H 1.20 ppm (d, *J* = 6.8 Hz, 3H) corresponding to three protons of the methyl group bonded to the secondary carbon at C-4. Two signals of two methyl groups in the methoxy group with δ_H 3.81 (s, 3H) and 3.82 (s, 3H) correlate with the C-3′ and C-4′ positions. In addition, two methylene groups resonate in doublet form at δ_H 1.80 (d, *J* = 7.0 Hz, 2H) and 1.31 (d, *J* = 7.0 Hz, 2H). A resonant signal at δ_H 5.03 (d, *J* = 10.3 Hz, 1H) was assigned to H-3 proton of olefin moiety. The remaining protons on the aromatic rings resonate in the 5.5–7.5 ppm range (see detail in Table 1).

The ^{13}C NMR spectrum of compound **1** shows that there are 22 resonance signals of the carbon atoms. In which, C atom of three methyl groups resonating at δ_C 29.71 ppm (C-3″) linked to C-4; carbons of two methoxy groups at 55.96 ppm (C-2″) and 56.00 ppm (C-1″) linked to C-3′ and C-4′ over an oxygen atom. The carbon atom at C-3 has a resonance signal at δ_C 93.79 ppm. The chemical shifts of the remaining carbon atoms are summarized in Table 1 depending on the HSQC, HMBC, and NOESY spectral data. Thus, structure **1** was identified as an undescribed compound from this plant depending on its NMR, HR-MS data, and comparison of the spectral data with its literature references [7,9] and it was named Capparisone A (Figure 1).

Compound **2** was obtained as a colorless amorphous and has a molecular ion peak in its MS high-resolution spectrum at *m/z* 369.0957 $[M+Na]^+$ (90%), the result of elemental analysis of compound **2** as C 62.24%, H 5.51%, and O 32.24%. Combined with NMR spectral data, compound **2** has the molecular formula as $C_{18}H_{18}O_7$ (Cal.

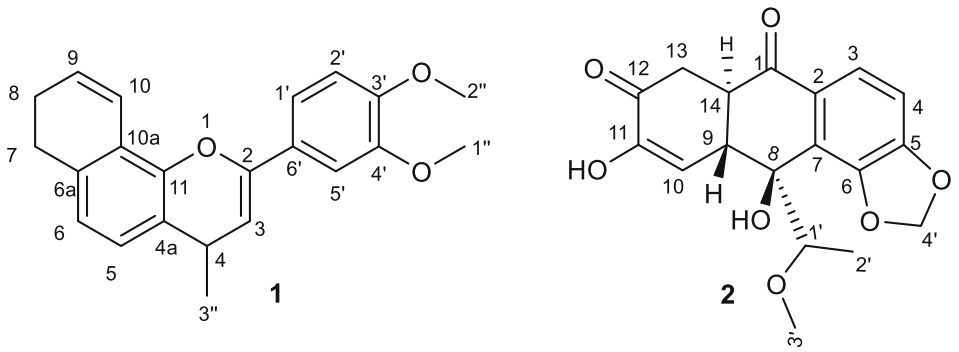

Figure 1: Structures of Capparisone A (**1**) and Capparisone B (**2**).

$C_{18}H_{18}O_7Na$ *m/z* 369.0950). The ^1H NMR spectrum of compound **2** showed a doublet signal at δ_H 0.99 ppm (d, J = 7.2 Hz, 3H) corresponding to three protons of the methyl group linked to the secondary carbon at C-1′. A signal at δ_H 2.52 (td, J = 7.4, 5.6 Hz, 1H) corresponds to the proton of the CH group at C-9. The resonance signals at 2.17 (dd, J = 14.7, 7.4 Hz, 1H) and 2.00 (dd, J = 14.7, 7.4 Hz, 1H) ppm are the resonance signals of two protons of the CH_2 group directly linked carbon atom sp^2 at C-13. The resonance signal of protons with a singlet signal at 5.88 (s, 2H) is two protons of the $-CH_2-$ group at C-4′ linked to two oxygen atoms. Other proton signals of **2** are summarized in Table 2.

The ^{13}C NMR spectra of substance **2** showed 18 resonance signals of carbon atoms. The carbon atom of group C═O at C-1 at δ_C 202.02 ppm, and the carbon atom at C-12 has δ_C 190.18 ppm. The methyl group linked to the secondary carbon has δ_C 16.94 ppm at C-2′. The chemical shift of the methylene group in the $-O-CH_2-O-$ group at C-4′ has δ_C 100.22 ppm. The oxygen-linked methyl group has a chemical shift of 54.60 ppm. The resonance signals of other carbon atoms are summarized in Table 2.

The HSQC and HMBC correlations of **2** show some important correlations between the proton signal and the resonance signal of the carbon atoms in the range of 2–4 bonds; protons of the methylene-linked methylene group ($-O-CH_2-O-$) interact with carbon C-5 and C-6; the methyl group of $-O-CH_3$ linked the C-1′ atom through the oxygen atom. The ^1H NMR spectrum of **2** exhibited H-9 with J = 7.4 and 5.6 Hz (<10 Hz) should be the configuration β [16,17] H-9 has no signal correlation with H-14, so

Table 3: Inhibitory activities of Capparisone A (**1**) and Capparisone B (**2**) from stems of *C. dongvanensis*

Compound	IC_{50} (μM)				
	HeLa	A549	Hep-G2	MCF7	RD
Capparisone A (**1**)	56.5 ± 1.2	47.6 ± 2.5	48.6 ± 3.5	25.1 ± 1.1	>100
Capparisone B (**2**)	85.1 ± 1.5	42.5 ± 1.1	37.4 ± 1.5	30.2 ± 1.2	65.2 ± 1.3
Ellipticine	1.5 ± 0.1	1.4 ± 0.2	0.7 ± 0.2	1.8 ± 0.3	1.9 ± 0.4

Table 4: DPPH free radical scavenging activities of Capparisone A (**1**), Capparisone B (**2**), and EA extract from stems of *C. dongvanensis*

Concentration (μg/mL)	% DPPH free radical scavenging			
	Capparisone A (**1**)	Capparisone B (**2**)	Ethyl acetate (EA) extract	Quercetin
40	22.4 ± 1.1	35.5 ± 1.0	23.4 ± 1.2	65.5 ± 1.2
80	29.5 ± 1.2	46.5 ± 1.1	30.5 ± 1.1	70.5 ± 1.2
160	35.5 ± 1.0	51.5 ± 1.1	35.5 ± 1.0	82.3 ± 1.1
320	60.4 ± 1.3	67.6 ± 1.2	43.5 ± 1.1	85.5 ± 1.1
640	65.5 ± 1.1	80.5 ± 1.0	52.5 ± 1.1	92.5 ± 1.3
1,280	85.6 ± 1.2	90.5 ± 1.2	63.5 ± 1.1	95.5 ± 1.2
EC_{50} (μg/mL)	597.5 ± 1.1	353.2 ± 1.1	1266.7 ± 1.1	17.8 ± 1.2

H-14 can exist as α-conjugation, H-9 is not correlated with H-1′ so the structural group at C-1′ can be in the form α-. Therefore, depending on the analysis data of **2** and comparison with literature, references [7–9] confirmed the structure of **2,** and it is an undescribed compound in the plant and is named Capparisone B (Figure 1).

3.2 Inhibitory activities

Inhibitory activities of compounds **1** and **2** were evaluated against five cancer cell lines HeLa A549, MCF7, RD, and Hep-G2 cancer cells using the experimental procedure described by Khang [24]. Ellipticine is a positive control. The results are summarized in Table 3.

The results indicated that compounds **1** and **2** were isolated from *C. dongvanensis* leaves and exhibited inhibitory activities against cancer cell lines with IC_{50} values in a ranking of 25.1–85.1 μM. The inhibition of Capparisone A (IC_{50} = 25.1 μM) on the MCF7 cell line has a better significance than its Capparisone B (IC_{50} = 30.2 μM) but less than its Ellipticine (IC_{50} = 1.8 μM). Otherwise, Capparisone A has no significant the inhibition on RD cell line.

3.3 DPPH free radical scavenging

The antioxidant capacity of compounds **1** and **2** and EA extract inhibit the free radical DPPH compared to the positive antioxidant control as Quercetin. The results are prsented in Table 4.

Two isolated compounds **1** and **2** and EA extract exhibit the anti-oxidant activity due to the reduction of the stable radical DPPH to 2,2-diphenyl-1-picrylhydrazine. Capparisone B has a better significant and active antioxidant capacity than the activities of Capparisone A, but less than the positive control (Quercetin). The EA extract also appeared that it has a significant antioxidant capacity (anti-DPPH) with EC50 as 1266.7 μg/mL less than the anti-DPPH activities of Capparisone A and B and each time the concentration of samples is increased, the percentage of inhibition of DPPH free radical is significantly increased [22].

4 Conclusions

The phytochemical investigation of the stem of *C. dongvanensis* (Capparaceae) plant is continued reporting. Novel polycyclic compounds Capparisone A (**1**) and B (**2**) were isolated and identified from *C. dongvanensis*. The inhibitory activities against five cancer cell lines and anti-DPPH reagents of Capparisone A (**1**) and B (**2**) show the possibility that these substances can guide further research for medical purposes.

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Conflict of interest: The authors declare no conflict of interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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