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Research Article

FIVE COMPOUNDS FROM *CERATOPHYLLUM DEMERSUM*

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ABSTRACT

*Ceratophyllum demersum is a traditional medicine used in Asian countries. This plant is native to Vietnam. This study was done to investigate the phytochemicals of plants of Ceratophyllum demersum grown in Lam Dong Province. Five compounds, cyclocolorenone (**1**), 1α-hydroxycyclocolorenone (**2**), lupeol (**3**), betullinic acid (**4**), and chakyunglupulin B (**5**) were isolated from the ethyl acetate extract of Ceratophyllum demersum. Their chemical structures were elucidated by comparing their spectroscopic data with previously reported data in the literature.*

***Keywords*:** 1α-hydroxycyclocolorenone; *Ceratophyllum demersum*; cyclocolorenone; betullinic acid; lupeol

1. Introduction

Ceratophyllum demersum is popularly distributed throughout ponds and rivers in China and Southeast Asia (Alzurfi et al., 2022; Lu et al., 2007; Qiming et al., 2006). In Vietnam, the plant is known by the local name "Rong duoi chon," grown widely in the Mekong Delta of South Vietnam. Different parts of *C. demersum* are widely used in traditional medicine. It has traditionally been used to treat diarrhea, fever, wounds, haemorrhoids or piles, intrinsic haemorrhages, hyperdipsia, and hematemesis (Li et al., 2020). Extracts, fractions, and isolated compounds of this plant had various bioactivities such as antibacterial (Ramesh, 2015), α-glucosidase inhibition (Li et al., 2020), antiviral, anticancer, antioxidant properties, antibacterial, antifungal, anticoagulant, anti-inflammatory, and inhibition activity against breast cancer cell line (Hoang et al., 2022). Up to now, phytochemical data of *C. demersum* revealed the presence of 21 compounds, including flavonoid glycosides, sterols, coumarin, and fatty acids (Alzurfi et al., 2022; Bankova et al., 1995; Lu et al., 2007; Qiming et al., 2006). However, little is known about the chemical data of the Vietnamese *C. demersum* plant. This paper reported the isolation

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and structural elucidation of five compounds from *C. demersum* collected in Lam Dong Province, Vietnam.

2. Experimental

2.1. General experimental procedures

The NMR spectra were recorded on a Bruker Avance spectrometer (500 MHz for ^{1}H–NMR and 125 MHz for ^{13}C–NMR) in acetone-d_6 and $CDCl_3$. Thin-layer chromatography was carried out on silica gel 60 (Merck, 40-63 μm), and spots were visualised by spraying with 10% H_2SO_4 solution, followed by heating.

2.2. Plant material

Ceratophyllum demersum was collected in Đuc Trong, Lam Đong, Vietnam, in October 2023. The scientific name of the material was identified as *Ceratophyllum demersum* by Assoc. Prof. Dr. Dang Van Son (Institute of Tropical Biology, Vietnam). A voucher specimen (No UEP-021) was deposited in the herbarium of the Department of Organic Chemistry, Faculty of Chemistry, Ho Chi Minh University of Education, Ho Chi Minh City, Vietnam.

2.3. Extraction and isolation

Dried and ground materials of *Ceratophyllum demersum* (5 kg) were extracted with EtOAc (30 L x 10, each day) at room temperature. The filtrated solution was evaporated at reduced pressure to obtain a crude extract (55.85 g). The **EA** extract was applied to silica gel column chromatography (CC) with a gradient system using the mobile phase as *n*-hexane: EtOAc (2.5:1-0:1, v/v) to afford 18 fractions (coded **EA1-EA18**). The fraction **EA2** (1.61 g) was further subjected to Sephadex LH-20 gel chromatography, with methanol as an eluent to give four fractions **EA2.1-EA2.4**. Fraction **EA2.1** (450 mg) was washed with acetone (50 mL x 3), leaving a solid (compound **3**, 200 mg). Next, the fraction **EA2.2** (0.87 g) was rechromatographed by a silica gel CC, eluted with *n*-hexane: EtOAc (10:1, v/v) to afford seven fractions S1-S7. Fraction **S4** (211 mg) was applied to silica gel CC, eluted with *n*-hexane: EtOAc (5:1, v/v) to afford compounds **1** (22 mg) and **2** (3.9 mg). Fraction **EA10** (2.88 g) was further subjected to Sephadex LH-20 gel chromatography and eluted with methanol to give five fractions **EA10.1-EA10.5**. The fraction **EA10.4** (0.59 g) was rechromatographed by silica gel CC, eluted with *n*-hexane: EtOAc (2:1, v/v) to afford three fractions **X1**-**X3**. Fraction **X2** (89 mg) was applied to silica gel CC, eluted with *n*-hexane: EtOAc (2:1, v/v) to afford compounds **4** (8.1 mg) and **5** (2.3 mg).

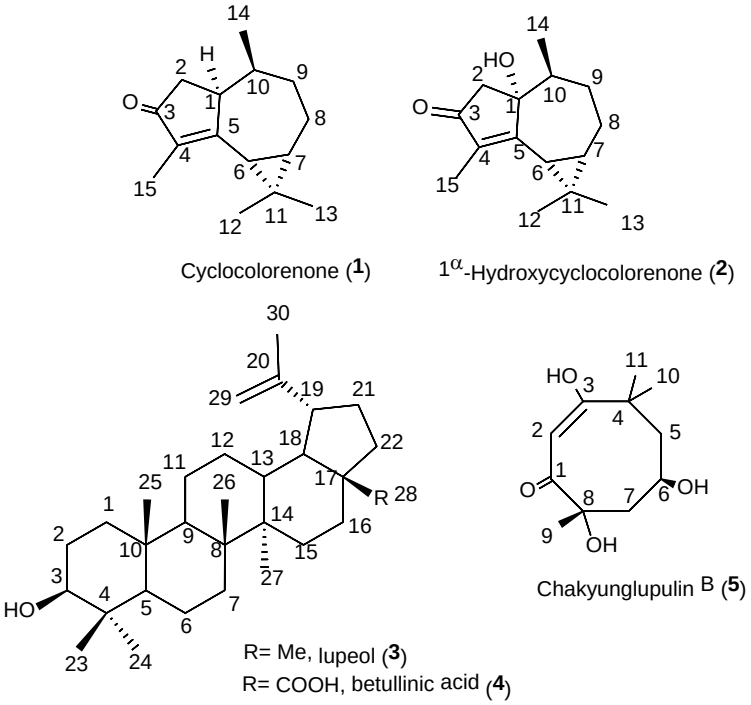

Figure 1. *Chemical structures of isolated compounds **1-5***

- **Cyclocolorenone** (**1**). Colourless oil. ^{1}H-NMR data (500 MHz, $CDCl_3$, δ ppm, *J* in Hertz): 2.96 (1H, *s*, H-1), 2.49 (1H, *dd*, *J* = 18.5, 6.5 Hz, H-2β), 2.05 (1H, *m*, H-2α), 2.01 (1H, *m*, H-8α), 2.01 (2H, *m*, H-8β), 1.98 (1H, *m*, H-7), 1.96 (1H, *m*, H-9β), 1.73 (3H, *s*, H-15), 1.63 (1H, *m*, H-9α), 1.48 (1H, *s*, H-6), 1.24 (3H, *s*, H-13), 1.15 (1H, *d*, *J* = 7.5 Hz, H-10), 1.02 (3H, *s*, H-12), 0.80 (3H, *d*, *J* = 7.0 Hz, H-14). ^{13}C-NMR data (125 MHz, $CDCl_3$, δ ppm): 42.4 (C-1), 40.1 (C-2), 207.2 (C-3), 132.9 (C-4), 176.5 (C-5), 28.4 (C-6), 31.6 (C-7), 32.3 (C-8), 21.0 (C-9), 32.2 (C-10), 25.9 (C-11), 29.4 (C-12), 16.4 (C-13), 17.3 (C-14), 14.0 (C-15).

- **1α-Hydroxycyclocolorenone** (**2**). Colorless oil. ^{1}H-NMR data (500 MHz, $CDCl_3$, δ ppm, *J* in Hertz): 2.96 (1H, *s*, H-1), 2.52 (1H, *m*, H-2β), 2.10 (1H, *m*, H-2α), 1.78 (3H, *s*, H-15), 1.48 (1H, *s*, H-6), 1.24 (3H, *s*, H-13), 1.15 (1H, *d*, *J* = 7.5 Hz, H-14), 1.03 (3H, *s*, H-12). ^{13}C-NMR data (125 MHz, $CDCl_3$, δ ppm): 74.9 (C-1), 45.2 (C-2), 207.5 (C-3), 133.3 (C-4), 177.0 (C-5), 28.5 (C-6), 31.1 (C-7), 32.8 (C-8), 21.5 (C-9), 45.9 (C-10), 27.5 (C-11), 29.9 (C-12), 17.8 (C-13), 18.7 (C-14), 9.8 (C-15).

- **Lupeol (3).** White amorphous powder. ^{1}H-NMR data ($CDCl_3$): 4.69 (1H, *d*, 2.0 Hz, H-29a), 4.56 (1H, *m*, H-29b), 3.19 (1H, *dd*, 11.4, 4.9 Hz, H-3) ,2.38 (1H, *td*, 11.1, 5.8 Hz, H-19), 1.68 (3H, *s*, H-30), 1.03 (3H, *s*, H-26), 0.97 (3H, *s*, H-23), 0.95 (3H, *s*, H-27), 0.83 (3H, *s*, H-25), 0.79 (3H, *s*, H-28), 0.76 (3H, *s*, H-24).

- **Betulinic acid (4)**. White amorphous powder. ^{1}H-NMR data (500 MHz, Acetone - d_6): 4.74 (1H, *s*, H-29α), 4.61 (1H, *s*, H-29β), 3.01 (1H, *m*, H-3), 1.69 (1H, *s*, H-30), 0.97 (3H, *s*, H-23),0.96 (3H, *s*, H-27), 0.82 (3H, *s*, H-25), 0.75 (3H, *s*, H-24).

- **Chakyunglupulin B (5)**. Yellow amorphous powder. ^{1}H-NMR data (500 MHz, Acetone-d_6, δ ppm, J in Hertz): 5.69 (1H, s, H-2), 4.33 (1H, m, H-6), 3.97 (3H, *s*, 7-OCH_3), 3.80 (3H, *s*, 3-OCH_3), 2.46 (1H, dt, J = 14.0, 3.0 Hz, H-5β), 1.97 (1H, dt, J = 14.5, 2.5 Hz, H-7β), 1.82 (3H, *s*, H-20), 1.78 (1H, m, H-5α), 1.78 (1H, s, H-11), 1.65 (3H, *s*, H-19), 1.53 (1H, dd, J = 14.5, 4.0 Hz, H-7α), 1.47 (1H, s, H-9), 1.27 (1H, s, H-10). ^{13}C-NMR (125 MHz, Acetone-d_6, δ ppm): 182.7 (C-1), 113.0 (C-2), 171.2 (C-3), 33.2 (C-4), 45.6 (C-5), 66.9 (C-6), 47.3 (C-7), 86.8 (C-8), 26.5 (C-9), 30.6 (C-10), 27.0 (C-11).

3. Results and discussion

Compound **1** was obtained as a colourless oil. The ^{1}H-NMR spectrum showed the presence of four methyls [δ 1.02 (3H, *s*, H_3-12), 1.24 (3H, *s*, H_3-13), 0.80 (3H, *d*, J = 7.0 Hz, H_3-14), 1.69 (3H, d, J = 2.0 Hz, H_3-15)], four methines [δ 2.96 (1H, *m*, H-1), 1.48 (1H, *s*, H-6), 1.98 (1H, *m*, H-7), 1.50 (1H, *m*, H-10)], and three methylenes [δ 2.05 (1H, *s*, H-2α), 2.49 (1H, *dd*, J = 18.5, 6.5 Hz, H-2β), 2.01 (1H, *m*, H-8α), 2.01 (1H, *m*, H-8β), 1.63 (1H, *m*, H-9α), 1.96 (1H, *m*, H-9β)]. The ^{13}C-NMR spectrum of **1** provided 15 carbons, including a carbonyl carbon (δ 207.2), three methylene carbons (δ 40.1, 32.3, and 21.0), four methyl carbons (δ 29.4, 16.4, 17.3, and 14.0), two olefinic carbons (δ 132.9 and 176.5), four methine carbons (δ 42.4, 28.4, 31.6, and 32.2), and one quaternary carbon (δ 25.9). HMBC correlations of the methyl H_3-12 with two methine carbons at δ_C 31.6 (C-7) and 28.4 (C-6), the quaternary carbon at δ_C 25.9 (C-11), and the methyl at δ_C 16.4 (C-13). Next, proton methine H-6 gave HMBC correlations with two olefinic carbons at δ_C 176.5 (C-5) and 132.9 (C-5). All the above spectroscopic data indicated that **1** was an aromadendrane-type sesquiterpene. A comparison of ^{1}H- and ^{13}C-NMR data of compound **1** with those of cyclocolorenone reported in the literature (Rao et al., 2004; Wu & Chen, 1992) revealed that compound **1** was elucidated as cyclocolorenone.

Compound **5** was obtained as a yellow amorphous powder. The ^{1}H-NMR spectrum showed the presence of four methyls [δ 1.47 (1H, *s*, H-9), 1.78 (1H, s, H-10), 1.27 (1H, *s*, H-11), 1.24 (1H, *s*, H-11)], and two methines [δ 5.69 (1H, s, H-2), 4.33 (1H, m, H-6)], and two methylenes [δ 1.78 (1H, m, H-5α), 2.46 (1H, dt, J = 14.0, 3.0 Hz, H-5β), 1.53 (1H, dd, J = 14.5, 4.0 Hz, H-7α), 1.97 (1H, dt, J = 14.5, 2.5 Hz, H-7β). The ^{13}C-NMR spectrum of **5** provided 11 carbons, including a carbonyl carbon (δ 182.7, C-1), two methine carbons (δ 113.0 and 66.9), a substituted sp2 carbon (δ 171.2), two quaternary carbons (δ 86.8 and 36.1), two methylene carbons (δ 45.6 and 47.3), and three methyl carbons (δ 26.5, 30.6, 27.0). Comparison of ^{1}H- and ^{13}C-NMR data of compound **5** with those of chakyunglupulin B reported in the literature (Kim et al., 2015). Thus, compound **5** was elucidated to be chakyunglupulin B.

To the best of our knowledge, this is the first time **1**-**5** were reported in *Ceratophyllum demersum* and the *Ceratophyllum* genus.

4. Conclusions

From *Ceratophyllum demersum* collected in Lam Dong province, five compounds *α*-cyclocolorenone (**1**), 1α-hydroxycyclocolorenone (**2**), lupeol (**3**), betullinic acid (**4**), and chakyunglupulin B (**5**) were isolated. Their chemical structures were determined by using NMR spectroscopic method and comparing them with the literature. To the best of our knowledge, this is the first time **1**-**5** were reported in *Ceratophyllum demersum* as well as the *Ceratophyllum* genus. Further studies on this species are in the progress.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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NĂM HỢP CHẤT TỪ CÂY RONG ĐUÔI CHỒN *CERATOPHYLLUM DEMERSUM*

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TÓM TẮT

*Ceratophyllum demersum được sử dụng trong y học cổ truyền ở nhiều nước châu Á. Đây là loài cây đặc hữu ở Việt Nam. Nghiên cứu về thành phần hóa học của cây rong đuôi chồn Ceratophyllum demersum thu hái ở Lâm Đồng được tiến hành. Từ cao ethyl acetate của Ceratophyllum demersum, thu hái tại Lâm Đồng, năm hợp chất bao gồm cyclocolorenone (**1**), 1α-hydroxycyclocolorenone (**2**), lupeol (**3**), betullinic acid (**4**), và chakyunglupulin B (**5**) được cô lập bằng các phương pháp sắc kí khác nhau. Cấu trúc hóa học của các hợp chất được xác định bằng các phương pháp phổ nghiệm đồng thời so sánh với các dữ liệu phổ trong tài liệu tham khảo.*

Từ khóa: 1α-hydroxycyclocolorenone; *Ceratophyllum demersum*; cyclocolorenone; betullinic acid; lupeol