ISSN 1859 - 4735

JOURNAL OF MEDICINAL MATERIALS

No. 5 - Vol. 30
9/2025

NATIONAL INSTITUTE OF MEDICINAL MATERIALS

3B QUANG TRUNG - CUA NAM - HANOI - VIETNAM
TEL: (+84-24) 38247058 FAX: (+84-24) 39348740
Email: tapchiduoclieu@nimm.org.vn; tapchiduoclieu@gmail.com

JOURNAL OF MEDICINAL MATERIALS (Print and Electronic Journals)

EDITORIAL BOARD (Term 2025-2030)

(Attached to Decision No. 666/QD-VDL dated April 24, 2025 signed by the Director of Vietnam National Institute of Medicinal Materials)

EDITOR-IN-CHIEF

Assoc. Prof. PhD. Do Thi Ha

I. Subcommittee of Pharmacognosy - Traditional Medicine

- **Assoc. Prof. Dr. Sci. Nguyen Minh Khoi - Head of Subcommittee**
- Assoc. Prof. PhD. Nguyen Thuong Dong - Member
- Prof. PhD. Nguyen Minh Duc - Member
- Prof. PhD. Tran Cong Luan - Member
- Prof. PhD. Won Keun Oh - Member
- Assoc. Prof. PhD. Tran Hung - Member
- Assoc. Prof. PhD. Nguyen Thi Bich Thu - Member
- Assoc. Prof. PhD. Phuong Thien Thuong - Member
- Assoc. Prof. PhD. Phan Minh Giang - Member
- Assoc. Prof. PhD. Le Nguyen Thanh - Member
- Assoc. Prof. PhD. Le Viet Dung - Member
- Assoc. Prof. PhD. Nguyen Quoc Huy - Member
- Assoc. Prof. PhD. Nguyen Thu Hang - Member
- PhD. Tran Minh Ngoc - Member
- PhD. Nguyen Van Tai - Member
- Assoc. Prof. PhD. Le Dinh Chi - Member
- PhD. Nguyen Tuan Hiep - Member.

II. Subcommittee of Pharmacology - Clinical Pharmacy

- **Assoc. Prof. PhD. Pham Thi Nguyet Hang - Head of Subcommittee**
- Assoc. Prof. Dr.Sci. Do Trung Dam - Member
- Assoc. Prof. PhD. Nguyen Thi Thu Huong - Member
- Assoc. PhD. William R. Folk - Member
- Assoc. Prof. PhD. Pham Thi Van Anh - Member
- Assoc. Prof. PhD. Nguyen Thuy Duong - Member
- Assoc. Prof. PhD. Le Van Minh - Member
- PhD. Tran Thi Hien - Member.

III. Subcommittee of Agriculture - Biology

- **PhD. Nguyen Van Khiem - Head of Subcommittee**
- PhD. Phan Thuy Hien - Member
- Assoc. Prof. PhD. Nguyen Van Tap - Member
- Assoc. Prof. PhD. Pham Thanh Huyen - Member
- Prof. PhD. Tran The Bach - Member.

IV. Subcommittee of Secretariat Administration

- **Assoc. Prof. PhD. Le Nguyen Thanh - Head of Subcommittee**
- PhD. Le Thi Xoan - Academic Secretary
- BSc. Nguyen Thi Minh Loc - Administrative Secretary.

CONTENTS VOL. 30(5), 2025
(Pages 305 - 384)

Journal of Medicinal Materials, 2025, Vol. 30, No. 5 (307 - 312)

ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF TRITERPENOIDS AND ALKALOIDS ISOLATED FROM *LYCOPODIELLA CERNUA* (L.) PIC. SERM.

Le Ba Vinh[1,2], Nguyen Thi Nga[3,4], Nguyen Cao Cuong[1], Nguyen Van Chinh[1], Nguyen Ngoc Linh[3,*]

[1]*Faculty of Medicine and Pharmacy, Yersin University of Da Lat, Lam Dong, Vietnam;*
[2]*Faculty of Science and Technology, University of Bergen, Bergen 5007, Norway;*
[3]*Institute of Medicine and Pharmacy, Thanh Do University, Hanoi, Vietnam;*
[4]*Faculty of Pharmacy, Hanoi University of Business and Technology, Hanoi, Vietnam*
*Corresponding author: nnlinh@thanhdouni.edu.vn

Received August 5th, 2025 Accepted August 26th, 2025

Summary

Lycopodiella cernua (L.) Pic. Serm., a perennial herb widely distributed across tropical and subtropical regions, has been traditionally used in Vietnamese and Chinese medicine for the treatment of hepatitis, inflammation, and musculoskeletal disorders. Despite its ethnopharmacological significance, comprehensive phytochemical and biological investigations of this species remain limited. In the present study, we report the isolation and structural elucidation of six secondary metabolites (**1-6**) from *L. cernua*, including alkaloids and triterpenoids. Their structures were determined through extensive spectroscopic analysis, including ^{1}H-NMR, ^{13}C-NMR, HSQC, and HMBC. Furthermore, the acetylcholinesterase (AChE) inhibitory activity of the isolated compounds was evaluated to assess their neuroprotective potential. These findings highlight the phytochemical diversity of *L. cernua* and underscore its value as a promising source of bioactive natural products with potential therapeutic applications.

Keywords: *Lycopodiella cernua*; *Triterpenoid; Alkaloid; Acetylcholinesterase inhibition; Alzheimer's disease.*

1. Introduction

The genus *Lycopodiella*, belonging to the family Lycopodiaceae, comprises a group of perennial herbaceous plants widely distributed across tropical and subtropical regions [1]. *Lycopodiella cernua* (L.) Pic. Serm., synonym *Palhinhaea cernua* (L.) Vasc. & Franco, commonly known as *Thông đất* in Vietnam, is frequently found at elevations up to 1,200 meters, particularly in open grasslands, rocky slopes, and abandoned lands near forest margins. Traditionally, *L. cernua* has been extensively used in Vietnamese and Chinese herbal medicine. All parts of the plant are utilized, primarily in decoction form, either alone or in combination with other medicinal herbs. In Vietnam, it is employed to treat acute hepatitis, conjunctivitis, rheumatism, chronic cough, and musculoskeletal pain [2]. Additionally, plant ash soaked in vinegar is traditionally applied topically to treat skin rashes. Phytochemical investigations of *Lycopodiella* species have revealed a rich diversity of bioactive compounds, particularly alkaloids, which are known for their complex polycyclic structures and diverse biological activities, including anti-inflammatory, antioxidant, and cytotoxic effects [3]. Previous studies on the chemical constituents of *L. cernua* have identified the presence of alkaloids, including representative members of the lycopodine, lycodine fawcettimine classes, as well as flavonoids, triterpenoids, and lignans [4]. Pharmacological evaluations have demonstrated that *L. cernua* exhibits notable bioactivities, such as cytotoxic, antioxidant, anti-inflammatory, xanthine oxidase inhibitory, and acetylcholinesterase (AChE) inhibitory effects [5]. Notably, these findings support its potential as a source of novel therapeutic agents targeting inflammation, oxidative stress, neurodegenerative diseases, and cancer. Despite its traditional medicinal use, comprehensive studies on the chemical composition and bioactive constituents of *L. cernua* are still scarce. Therefore, this research focuses on the isolation of secondary metabolites and the assessment of AChE inhibitory activity to gain further insight into its neuroprotective potential.

2. Materials and methods

2.1. General experimental procedures

Open-column chromatography was performed using *silica gel* (Kieselgel 60, 70-230 mesh and

230-400 mesh, Merck, Darmstadt, Germany) and reversed-phase YMC*GEL ODS-A (12 nm, S-150 μm, YMC Co., Ltd., Japan). Thin-layer chromatography (TLC) was performed on Merck precoated *silica gel* 60 F_{254} (1.05554.0001) and RP-C_{18} $F_{254}S$ plates (1.15685.0001) to monitor the separation process. Spots were visualized under UV light (254 and 365 nm) and by spraying with 10 % (*v/v*) aqueous H_2SO_4, followed by heating. All solvents and reagents used were of HPLC grade and purchased from Aldrich Chemical Co. (Saint Louis, Missouri, USA). NMR spectra (^1H, ^{13}C, and 2D) were recorded on a Bruker Avance III 600 MHz spectrometer. Preparative HPLC was conducted using a Younglin 9000 gradient HPLC system equipped with a Shiseido C_{18} column (21.2 × 150 mm, 5 μm). The flow rate was 5 mL/min.

2.2. Plant Material

The whole plant sample of *L. cernua* was collected from Lam Dong in September 2024. One of the authors, Dr. Nguyen Cao Cuong, verified the plant's authenticity (*Lycopodiella cernua* (L.) Pic. Serm.), and a voucher specimen (MTD 002) is now housed in the herbarium of the Yersin University of Da Lat, Faculty of Medicine and Pharmacy, Lam Dong, and Institute of Pharmacy, Thanhdo University, Vietnam.

2.3. Extraction and isolation

The dried whole plant of *L. cernua* (3.5 kg) was ground into a fine powder and extracted with 80% ethanol by maceration at room temperature (3 × 10 L) for 24 hours. The ethanol extract was filtered and concentrated under reduced pressure to yield 170 g of crude extract. The crude extract was suspended in 4 L of water and successively partitioned with *n*-hexane and ethyl acetate to afford *n*-hexane, ethyl acetate (EtOAc), and water (W) extracts. The EtOAc extract (80.5 g) was fractionated into five subfractions (E1-E5) by *silica gel* column chromatography using a *n*-hexane-EtOAc gradient (from 2:1 to 1:1, *v/v*). Fraction E3 (30 g) was further separated by *silica gel* column chromatography with an *n*-hexane-EtOAc gradient (20:1 to 1:1, *v/v*), producing four subfractions (E3A-E3D). Subfraction E3B (7.0 g) was purified by reversed-phase chromatography on an RP-C_{18} column using acetone-water (4:3, *v/v*) as the mobile phase, yielding compounds **6** (3.5 mg) and **2** (10.5 mg). Subfraction E3C (14.0 g) was subjected to reversed-phase C_{18} column chromatography using CH_3OH-water (1:1, *v/v*) and further purified by HPLC with a gradient of acetonitrile-water from 80:20 to 100% acetonitrile, yielding compounds **5** (10.0 mg), **1** (2.0 mg), and **3** (7.0 mg). Fraction E4 (15 g) was separated by *silica gel* column chromatography with an *n*-hexane-ethyl acetate gradient (6:1 to 1:1, *v/v*), yielding four subfractions (E4A-E4D). Finally, subfraction E4A (1.4 g) was purified by reversed-phase chromatography on an RP-C_{18} column with methanol/water (8:1, *v/v*), followed by Sephadex™ LH-20 column chromatography using CH_3OH-water (1:1, *v/v*), affording compound **4** (10.0 mg).

Compound **1**: White powder, ^1H NMR (600 MHz, CD_3OD): δ_H 3.65 (1H, m, H-5), 3.25 (1H, m, H-7), 5.45 (1H, dd, J = 3.0, 12.0 Hz, H-9), 3.09 (1H, m, H-13), 0.90 (3H, d, J = 6.5 Hz, H-16). ^{13}C NMR (150 MHz, CD_3OD): δ_C 171.1 (C-1), 33.6 (C-2), 19.9 (C-3), 31.2 (C-4), 52.0 (C-5), 41.6 (C-6), 47.5(C-7), 42.5 (C-8), 68.7 (C-9), 21.5 (C-10), 25.5 (C-11), 23.4 (C-12), 59.2 (C-13), 39.9 (C-14), 26.3 (C-15), 22.5 (C-16).

Compound **2**: White powder, ^1H NMR (600 MHz, CD_3OD): δ_H 5.42 (1H, dd, J = 2.5, 12.0 Hz, H-9), 3.75 (1H, d, J = 2.5 Hz, H-12), 2.89 (1H, d, J = 7.0 Hz, H-13), 0.88 (3H, d, J = 6.5 Hz, H-16). ^{13}C NMR (150 MHz, CD_3OD): δ_C 171.2 (C-1), 33.7 (C-2), 19.8 (C-3), 31.1 (C-4), 52.3 (C-5), 42.2 (C-6), 50.4 (C-7), 42.3 (C-8), 68.7 (C-9), 17.5 (C-10), 34.0 (C-11), 70.6 (C-12), 60.3 (C-13), 38.8 (C-14), 26.8 (C-15), 23.1 (C-16).

Compound **3**: White amorphous powder, ^1H (600 MHz, DMSO-d_6) and ^{13}C-NMR (150 MHz, DMSO-d_6): see **Table 1**.

Compound **4**: White amorphous powder, ^1H (600 MHz, DMSO-d_6) and ^{13}C-NMR (150 MHz, DMSO-d_6): see **Table 1**.

Compound **5**: White amorphous powder, ^1H (600 MHz, DMSO-d_6) and ^{13}C-NMR (150 MHz, DMSO-d_6): see **Table 1**.

Compound **6**: White amorphous powder, ^1H (600 MHz, DMSO-d_6) and ^{13}C-NMR (150 MHz, DMSO-d_6): see **Table 1**.

2.4. In vitro AChE inhibitory activity

The *in vitro* AChE inhibitory activities of the isolated compounds were evaluated based on previously reported methods [6],[7].

3. Results and discussion

The EtOH extract of *L. cernua* was subjected to successive solvent partitioning using *n*-hexane and EtOAc in order of increasing polarity. Subsequent purification of six compounds (**1-6**) was accomplished through a series of chromatographic techniques, including repeated

silica gel, reversed-phase C_{18} (RP-C_{18}), and Sephadex LH-20 column chromatography, as well as high-performance liquid chromatography (prep-HPLC).

Compound **1** was obtained as a white powder. It gave a positive reaction with Dragendorff's reagent, indicating that it is an alkaloid—a class of compounds commonly found in the genus *Lycopodiella*. The ^{1}H-NMR spectrum revealed a methyl doublet at δ_H 0.90 (d, J = 6.5 Hz), along with methine proton signals attached to nitrogen atoms at δ_H 3.65 (m, H-5), 3.25 (m, H-7), 5.45 (dd, J = 3.0, 12.0 Hz, H-9), and 3.09 (m, H-13). The ^{13}C-NMR and HSQC spectra showed 16 carbon signals, including one methyl group (δ_C 22.5, C-16), nine methylene groups (δ_C 42.5, 41.7, 40.0, 33.7, 31.2, 25.6, 23.5, 21.6, and 19.9), five methine groups (δ_C 68.7, 59.2, 52.1, 47.5, and 26.3), and one quaternary carbon. A signal at δ_C 171.2 indicated the presence of a carbonyl group. The ^{1}H- and ^{13}C-NMR data suggest that compound **1** is a cernuane-type alkaloid with a tetracyclic skeleton containing two nitrogen atoms (Fig. 1). HMBC correlations from H-16 (δ_H 0.90) to C-8 (δ_C 42.5), C-14 (δ_C 40.0), and C-15 (δ_C 26.3) confirm that the methyl group is attached at the C-15 position. Based on combined 1D and 2D NMR data, including HSQC and HMBC spectra (**Fig. 2**), compound **1** was unambiguously identified as deoxylycocernuine [8], and its relative configuration was determined from the corresponding NMR data.

Compound **2** also gave a positive reaction with Dragendorff's reagent, indicating that it is an alkaloid. The NMR data of compound **2** are very similar to those of compound **1**, except for a minor chemical shift at C-12, suggesting the presence of a hydroxyl group attached to C-12 (Fig. 1). This was confirmed by HMBC correlations from H-14 (δ_H 1.45 (m) and 1.83 (m)) to C-13 (δ_C 60.3) and C-12 (δ_C 70.7). The relative configuration at the C-12 position was identified as α, based on the agreement of the NMR data with lycocernuine [8]. Therefore, compound **2** was identified as lycocernuine [8].

Compound **3** was isolated as a white amorphous powder. The NMR data of compound **3** indicated that it belongs to the serratane-type triterpenoids, which are major secondary metabolites previously identified in *L. cernua* [5]. The ^{1}H-NMR spectrum revealed six singlet signals for tertiary methyl groups (each 3H, s) at δ_H 1.05 (CH_3-23), 0.73 (CH_3-25), 0.77 (CH_3-26), 0.60 (CH_3-23), 0.79 (CH_3-29), and 0.81 (CH_3-30), one olefinic proton at δ_H 5.29 (1H, br s, H-15); two oxygenated methine protons at δ_H 3.22 (1H, br s, H-21) and 3.14 (1H, dd, J = 5.0, 13.0, H-3); and a hydroxymethylene group appearing at δ_H 3.26 (1H, d, J = 11.0 Hz, H-24a), and 3.80 (1H, d, J = 11.0 Hz, H-24b). Detailed analysis of the ^{13}C-NMR and HSQC spectra revealed 30 carbon signals, including two olefinic carbons at δ_C 138.1 (C-14) and 121.9 (C-15), two oxygenated methines at δ_C 78.5 (C-3) and 73.8 (C-21), one oxymethylene carbon at δ_C 62.9 (C-24), and and a characteristic methylene carbon of the serratane skeleton at δ_C 56.9 (C-27) [9]. This was further supported by detailed analysis of HMBC correlations, which clearly established the planar structure of compound **3**. Notable HMBC cross-peaks were observed from H-23 (δ_H 1.05) and H-24 (δ_H 3.26 and 3.80) to C-3 (δ_C 78.5) and C-4 (δ_C 42.3). Additionally, HMBC correlations from H-30 (δ_H 0.81) to C-21 (δ_C 73.8) and C-22 (δ_C 37.0) were also clearly detected (**Fig. 2**). The relative configuration at C-3 and C-21 of compound **3** was determined by comparing its ^{1}H and ^{13}C-NMR data with those of previously reported serratane-type triterpenoid isomers, serratendiol and 21-*epi*-serratendiol [10]. The detailed chemical shift of C-3 (δ_C 78.5) and C-21 (δ_C 73.8) of **3** verified that the relative configuration of the hydroxyl group at C-3 and C-21 was β configuration. Therefore, compound **3** was identified as 3-*epi*-lycoclavanol [11].

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of **3-6** (^{1}H: 600 MHz; ^{13}C: 150 MHz)

	3		**4**		**5**		**6**	
Position	δ_C	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)
1	38.1	0.88 s/1.69 m	38.6	0.92 m/1.80 m	33.1	1.30 m/1.35 m	35.0	1.37 m/1.60 m
2	27.3	1.60 m/1.66 m	28.1	1.58 s/1.98 m	25.5	1.40 m/1.78 m	27.8	1.34 m/2.11 m
3	78.5	3.14 dd (5.5; 13.0)	76.5	2.99 dd (4.5; 12.0)	68.2	3.56 d 3.5)	71.4	3.99 br s
4	42.3	-	48.1	-	42.7	-	49.0	-
5	55.7	0.80 m	55.6	0.92 m	49.2	1.31 m	50.2	1.60 m
6	19.1	1.42 m/1.47 m	20.1	1.63 m/1.78 m	18.6	1.35 m	21.3	1.68 m/1.96 m
7	45.2	1.15 m/1.30 m	44.7	1.11 s/1.32 m	45.2	1.11 m/1.28 m	46.2	1.31 m/1.51 m
8	37.5	-	36.5	-	36.9	-	39.0	-

	3		**4**		**5**		**6**	
Position	δ_C	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)
9	62.2	1.76 m	61.6	0.74 s	62.3	0.84 m	63.2	1.08 d (2.5)
10	36.7	-	38.0	-	37.6	-	39.7	-
11	24.7	1.00 m/1.65 m	24.9	0.98 m/1.70 m	24.7	1.00 m/1.65 m	25.6	1.63 m/1.88 s
12	26.9	1.10 m/1.97 m	27.0	1.11 s/1.99 m	26.8	1.06 m/2.00 m	27.4	1.59 m /2.11 m
13	56.4	1.79 m	56.5	1.80 m	56.5	1.80 m	60.2	2.53 m
14	138.1	-	137.9	-	138.2	-	167.4	-
15	121.9	5.29 br s	122.1	5.27 br s	121.8	5.29 br s	128.9	5.70 br s
16	23.6	1.78 m/1.87 m	23.3	1.80 m/1.89 m	23.5	1.8 m/1.91 m	204.2	-
17	42.7	1.60 m	43.5	1.69 m	42.8	1.61 m	60.2	2,78 s
18	35.5	-	35.3	-	35.4	-	45.6	-
19	30.8	1.43 m	30.7	1.42 m	30.8	1.42 m/1.80 m	32.3	1.60 m/1.85 m
20	25.4	1.45 m/1.79 m	25.3	1.42 m/1.72 m	25.4	1.40 m/1.78 m	26.3	1.31 m/1.96 m
21	73.8	3.22 br s	68.0	3.66 s	73.8	3.22 br s	71.3	4.00 d (1.5)
22	37.0	-	42.0	-	37.0	-	43.7	-
23	22.9	1.05 s	23.8	1.25 s	22.8	0.87 s	24.6	1.24 s
24	62.9	3.26 d (11.0)/ 3.80 d (11.0)	178.0	-	64.0	3.18 dd (5.5, 10.5)/ 3.42 dd (5.5, 10.5)	179.3	-
25	15.8	0.73 s	13.6	0.71 s	16.0	0.72 s	13.9	0.71 s
26	19.6	0.77 s	19.3	0.79 s	19.6	0.77 s	19.9	0.95 s
27	55.9	1.67 m/2.18 m	55.7	1.66 m/2.17 d (14.5)	55.9	1.64 d (15.0)/2.20 d (15.0)	57.1	2.01 d (15.0)/ 2.56 d (15.0)
28	13.0	0.60 s	14.0	0.58 s	13.0	0.62 s	15.8	0.79 s
29	21.6	0.79 s	63.4	3.28 d (11.0)/3.49 d (11.0)	21.6	0.80 s	64.8	3.58 d (11.0)/4.09 d (11.0)
30	28.0	0.81 s	22.0	0.87 s	28.0	0.81 s	22.3	1.28 s
3-OH	-	4.94 d (4.5)	-	4.18 s	-	4.00 d (4.5)		
21-OH	-	4.20 d (4.0)	-	4.05 s	-	4.19 d (4.0)		
OCH_3							51.6	3.65 s

The signal assignments were determined based on 1D and 2D NMR, including HSQC and HMBC experiments.

The ^{1}H NMR spectrum of compound **4** revealed proton signals corresponding to five tertiary methyl groups at δ_H 1.25 (s, H_3-23), 0.71 (s, H_3-25), 0.79 (s, H_3-26), 0.58 (s, H_3-28), and 0.87 (s, H_3-30). An olefinic proton was observed at δ_H 5.27 (1H, br s, H-15), along with two oxymethine protons at δ_H 3.66 (1H, br s, H-21) and 2.99 (1H, dd, J = 4.5, 12.0 Hz, H-3). In addition, signals corresponding to a hydroxymethylene group were detected at δ_H 3.28 (1H, d, J = 11.0 Hz, H-29). Analysis of the ^{13}C NMR and HSQC spectra revealed the presence of 30 carbon atoms, including two olefinic carbons at δ_C 137.9 (C-14) and 122.1 (C-15); two oxymethine carbons at δ_C 76.5 (C-3) and 68.0 (C-21); one oxymethylene carbon at δ_C 63.4 (C-29); a carboxylic acid carbon at δ_C 178.0 (C-24); and a characteristic serratane-type methylene carbon at δ_C 55.7 (C-27). Similar to compound **3**, HMBC correlations confirmed the planar structure of compound **4** (**Fig. 2**). Notable HMBC cross-peaks were observed from H-23 (δ_H 1.25) to C-24 (δ_C 178.0), C-3 (δ_C 76.5), and C-4 (δ_C 48.1). Additionally, HMBC correlations from H-30 (δ_H 0.87) to C-21 (δ_C 68.0) and C-22 (δ_C 42.0) were clearly identified. The relative configuration of compound **4** was determined based on the agreement of its NMR spectral data with lycernuic acid B [12] and is presented in **Table 1**. Based on these spectral data, compound **4** was identified as 3*β*,21*β*,29-trihydroxyserrat-14-ene-24-oic acid [12].

Compound **5** was obtained as a white amorphous powder. Its structure is closely related to that of compound **3** (3-*epi*-lycoclavanol), with the only significant difference observed at the C-3 position. In compound **3**, the C-3 carbon resonates at δ_C 78.5, whereas in compound **5**, the chemical shift for C-3 is observed at δ_C 68.2, indicating a change in stereochemistry. The relative configuration of the hydroxyl groups at C-3 and C-21 was determined by comparing the characteristic coupling constants with those reported in **Table 1**. The ^{1}H NMR signals at δ_H 3.56 (d, J = 3.5 Hz, H-3) and δ_H 3.22 (br s, H-21) support the assignment of an *α*-orientation for the hydroxyl group at C-3 and a *β*-orientation at C-21. Based on these data, compound **5** was identified as 3*α*,21*β*,24-trihydroxyserrat-14-ene [10].

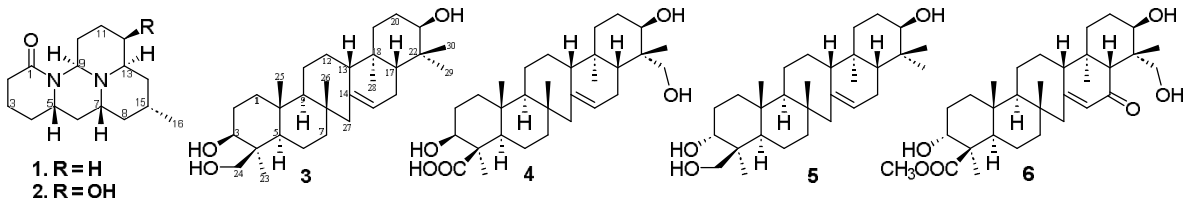

Fig. 1. Structures of isolated compounds (**1-5**) from *L. cernua*

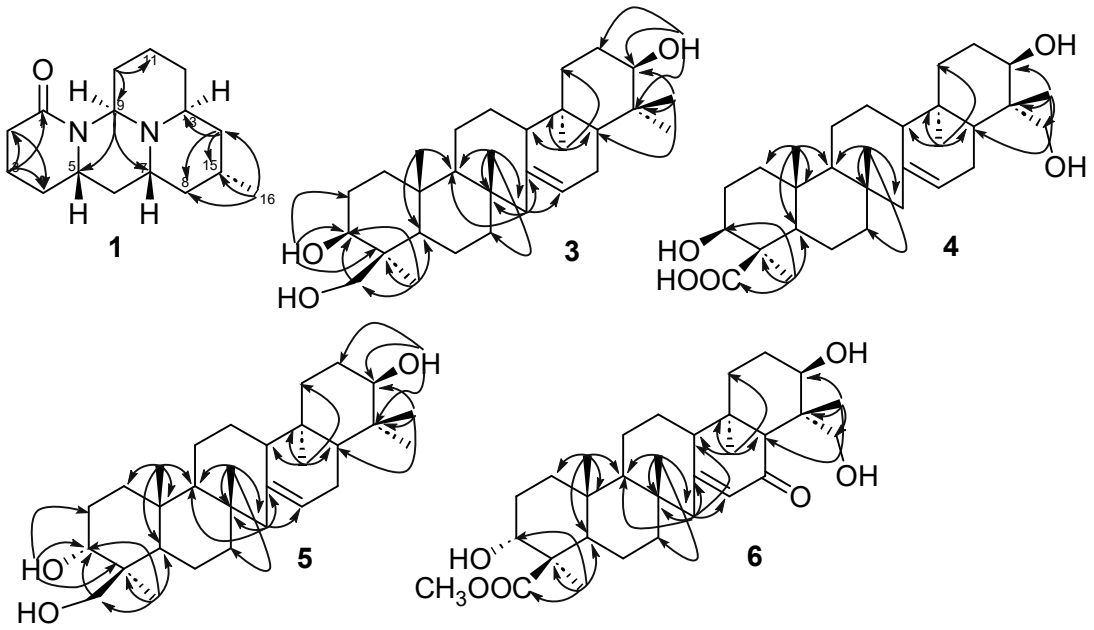

Fig. 2. Important HMBC cross-peak of compounds **1**, and **3-6**

Compound **6** was also obtained as a white amorphous powder. Its NMR data were generally similar to those of compound **4**, with notable differences observed at the C-3, C-16, and C-24 positions. The chemical shifts of C-3 and C-16 in compound **6** were δ_C 71.4 and 204.2, respectively, suggesting a difference in the stereochemistry at C-3 and the presence of a carbonyl group at C-16. Furthermore, this assignment is supported by a key HMBC correlation between the methoxy proton signal at δ_H 3.65 (OCH_3) and the carbonyl carbon at δ_C 179.3 (C-24), confirming the presence of a carbomethoxy group attached at C-24 (**Fig. 2**). The configurations of the hydroxyl groups at C-3 and C-21 were assigned as $\alpha$and β, respectively, based on characteristic coupling patterns observed in the ^{1}H NMR spectrum: δ_H 3.99 (br s, H-3) and δ_H 3.82 (d, J = 1.5 Hz, H-21), consistent with previously reported data (**Table 1**). Detailed analysis of the HSQC and HMBC spectra further confirmed that compound **6** is identical to lycernuic ketone B [12].

Acetylcholinesterase (AChE) is a crucial enzyme in the cholinergic nervous system, primarily responsible for the rapid hydrolysis of the neurotransmitter acetylcholine into choline and acetate. This enzymatic action terminates synaptic transmission at cholinergic synapses, thereby regulating neural signal intensity and duration. Inhibition of AChE leads to elevated acetylcholine levels in the synaptic cleft, which is a key mechanism exploited in the treatment of neurodegenerative diseases such as Alzheimer's disease. Consequently, AChE is an important pharmacological target in both neuroscience and drug development [13].

With slight modifications, the *in vitro* AChE inhibitory activity was evaluated following our previously established protocol [6],[7]. To assess the inhibitory effects of compounds **1-6** on AChE, the enzyme conversion rate was measured in the presence of each compound. Initially, all compounds were screened at a concentration of 100 μM. Among them,

compound **1** exhibited the strongest inhibitory activity against AChE, with an IC_{50} value of 1.2 ± 0.3 μM—comparable to that of the reference inhibitor tacrine (IC_{50} = 125.6 ± 1.1 nM). Compound **2** also showed significant AChE inhibitory activity, with an IC_{50} value of 1.7 ± 0.2 μM. The remaining compounds (**3-6**) displayed IC_{50} values greater than 100 μM, indicating a lack of notable AChE inhibition for these triterpenoids. The results suggest that alkaloids derived from *L. cernua* may play a crucial role in the inhibition of AChE, an enzyme intimately linked to the pathogenesis of Alzheimer's disease. Previously, our study identified two phenolics—methyl ferulate and methyl *p*-coumarate—with notable antioxidant potential, and one triterpenoid, tohogenol, exhibiting significant AChE inhibitory activity, thereby underscoring the therapeutic promise of *L. clavatum* in neuroprotective strategies [6]. Notably, this is also the first report to contribute two previously unreported AChE inhibitory agents, **1-2** from *L. cernua*, further enriching the phytochemical and pharmacological profile of this medicinal plant.

4. Conclusion

In conclusion, chemical investigation of the ethanol extract from the whole plant *L. cernua* yielded six compounds, including two alkaloids and four triterpenoids: deoxylycocernuine (**1**), lycocernuine (**2**), 3-*epi*-lycoclavanol (**3**), 3*β*,21*β*,29-trihydroxyserrat-14-ene-24-oic acid (**4**), lycoclavanol (**5**), and lycernuic ketone B (**6**). The AChE inhibitory activities of the isolated compounds were evaluated, revealing that compounds **1** and **2** exhibited notable IC_{50} values of 1.2 ± 0.3 μM and 1.7 ± 0.2 μM, respectively. This is the first report identifying compounds **1** and **2** as AChE inhibitors. The results suggest that alkaloids from *L. cernua* are responsible for the observed inhibition of AChE. These findings indicate that the alkaloids isolated from the whole plant of *L. cernua* exhibit promising pharmacological properties, potentially contributing to therapeutic strategies for neurodegenerative disorders.

References

1. Joshi R. K. (2023), Bioactive usual and unusual triterpenoids derived from natural sources used in traditional medicine. *Chemistry & Biodiversity*, 20(2), e202200853.

2. Chuong N. N., Trung B. H., Luan T. C., Hung T. M., Dang N. H., Dat N. T. (2014), Anti-amnesic effect of alkaloid fraction from *Lycopodiella cernua* (L.) Pic. Serm. on scopolamine-induced memory impairment in mice. *Neuroscience Letters*, 575(42-46).

3. Giang V. H., Thuy L. T., Cham P. T., Vinh L. B., Ban N. K., Linh T. M., Mai N. C., Hoe P. T., Huong T. T., Dang N. H. (2022), Chemical constituents from *Lycopodiella cernua* and their anti-inflammatory and cytotoxic activities. *Natural Product Research*, 36(16), 4045-4051.

4. Hung T. M., Lee J. S., Chuong N. N., Kim J. A., Oh S. H., Woo M. H., Choi J. S., Min B. S. (2015), Kinetics and molecular docking studies of cholinesterase inhibitors derived from water layer of *Lycopodiella cernua* (L.) Pic. Serm.(II). *Chemico-Biological Interactions*, 240(74-82).

5. To D. C., Tran M. H., Oh S. H., Kim J. A., Ali M. Y., Woo M. H., Choi J. S., Min B. S. (2015), Isolation of cholinesterase and β-secretase 1 inhibiting compounds from *Lycopodiella cernua*. *Bioorganic & Medicinal Chemistry*, 23(13), 3126-3134.

6. Linh N. N., Thuy V. T. T., Cuong N. C., Vinh L. B. (2025), Serratane triterpenoids and caffeic acid derivatives from whole plants of *Lycopodium clavatum* and their potential DPPH and acetylcholinesterase inhibitory activities. *Journal of Medicinal Materials*, 30(2), 107-112.

7. Vinh L. B., Dan G., Phong N. V., Cho K., Kim Y. H., Yang S. Y. (2021), *In vitro* investigation of acetylcholinesterase inhibitors isolated from the fruit of *Stauntonia hexaphylla*. *Chemistry of Natural Compounds*, 57(784-787).

8. Morita H., Hirasawa Y., Shinzato T., Kobayashi J. I. (2004), New phlegmarane-type, cernuane-type, and quinolizidine alkaloids from two species of *Lycopodium*. *Tetrahedron*, 60(33), 7015-7023.

9. Li J., Xu P. S., Tan L. H., Zou Z. X., Wang Y. K., Long H. P., Zhou G., Li G., Xu K. P., Tan G. S. (2017), Neolignans and serratane triterpenoids with inhibitory effects on xanthine oxidase from *Palhinhaea cernua*. *Fitoterapia*, 119(45-50).

10. Seto H., Furihata K., Guangyi X., Xiong C., Deji P. (1988), Assignments of the 1H-and 13C-NMR spectra of four lycopodium triterpenoids by the application of a new two-dimensional technique, heteronuclear multiple bond connectivity (HMBC). *Agricultural and Biological Chemistry*, 52(7), 1797-1801.

11. Liang L. F., Chen Q. H., Xu J. H., Liu T., Song X. N., Chen H. Y., Chen H. (2019), Serratanes from whole plant of *Palhinhaea cernua*. *Chemistry of Natural Compounds*, 55(4), 759-761.

12. Zhang Z., ElSohly H. N., Jacob M. R., Pasco D. S., Walker L. A., Clark A. M. (2002), Natural products inhibiting Candida albicans secreted aspartic proteases from *Lycopodium cernuum*. *Journal of Natural Products*, 65(7), 979-985.

13. Hossain M. S., Hussain M. H. (2025), Multi-target drug design in Alzheimer's disease treatment: Emerging technologies, advantages, challenges, and limitations. *Pharmacology Research and Perspectives*, 13(4), e70131.