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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH ARTICLE

A new cadinane sesquiterpenoid from *Eupatorium adenophorum* and *α*-glycosidase and AChE inhibitory activities of a gossypetin acylglucoside

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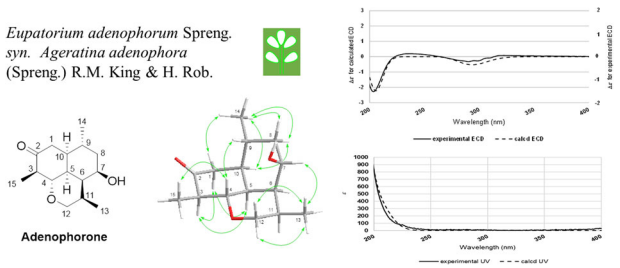
Abstract

To investigate the chemical constituents of *Eupatorium adenophorum* Spreng. (*syn. Ageratina adenophora* (Spreng.) R.M. King & H. Rob.) growing in Vietnam, the water-soluble fraction from the leaf methanol extract was fractionated by column chromatography. A new sesquiterpenoid, named adenophorone (**1**), was isolated along with 11 known compounds (**2**-**12**). The interpretation of HR-MS and 1D and 2D NMR spectroscopic data together with experimental and theoretical ECD calculations established the absolute stereostructure of compound **1**. Two isolated flavonol glucosides **9** and **10** were subjected to enzyme inhibition assays. Gossypetin 5-*O*-(6″-(*E*)-caffeoyl)-*β*-D-glucopyranoside (**9**) inhibited *α*-glycosidase activity with an IC_{50} value of 24.0 ± 1.61 μg/mL (80% inhibition at 256 μg/mL) and acetylcholinesterase activity with an IC_{50} value of 217.60 ± 15.47 μg/mL (54% inhibition at 256 μg/mL). Quercetagetin 7-*O*-*β*-D-glucopyranoside (**10**) slightly inhibited the two enzymes (27% and 34% inhibition, respectively) at the maximal tested concentration of 256 μg/mL. The present study is the first report on the chemical constituents of the water-soluble fraction of *E. adenophorum*. The study also provides some evidence for the *α*-glycosidase and acetylcholinesterase activities of the rare polyhydroxyflavonol acylglycoside gossypetin 5-*O*-(6″-(*E*)-caffeoyl)-*β*-D-glucopyranoside.



Keywords Acetylcholinesterase · Adenophorone · *α*-Glycosidase · Cadinane · *Eupatorium adenophorum*

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Introduction

Eupatorium is a large plant genus of the family Asteraceae, which is distributed throughout tropical America, Europe, Africa, and Asia [1]. The genus *Eupatorium* has been known for its therapeutic properties for many decades and is a promising bioresource for bioactive substances for drug development [2]. In Vietnam, Nguyen T. B. and Pham H. H. recorded twelve *Eupatorium* species, including *E. odoratum* L., *E. triplinerve* Vahl., *E. reesesii* Wall., *E. quaternum* DC., *E. cannabium* L., *E. capillifolium* (Lamk.) Small, *E. chinense* L., *E. coelestinum* L., *E. fortunei* Turcz., *E. heterophyllum* DC., *E. japonicum* Thunb., and *E. lindleyyanum* DC. [3, 4]. *E. adenophorum* Spreng. [*syn. Ageratina adenophora* (Spreng.) R.M. King & H. Rob.] is a perennial shrub growing 1–2 m in height, which is considered a harmful weed of crops and the natural environment. Originating from Mexico, *E. adenophorum* has spread to Vietnam, China, and adjacent regions. In Vietnam, *E. adenophorum* is included in the List of Invasive Plant Species of Vietnam according to Circular 35/2018/TT-BTNM issued by the Ministry of Natural Resources and Environment of Vietnam in 2018. The leaves of *E. adenophorum* are used in traditional medicine in China, India, and Nigeria [5–8]. In eastern Himalayas young leaves and shoots of *E. adenophorum* are given orally against dysentery [5]. In Nigeria the leaves of *E. adenophorum* are used locally to treat fever, diabetes, and inflammation [6]. The root juice of *E. adenophorum* is recommended to treat fever [7, 8]. In China, *E. adenophorum* has been used as a traditional Chinese medicine to treat fever, desinsectization, traumatism, and phyma [8]. Phytochemistry and biological activities of leaf extracts and isolates of *E. adenophorum* have been described in several reviews and in a growing number of publications. The presence of cadinane sesquiterpenes [9–11], quinic acid derivatives [9, 10, 12], flavonoids [9], and phenolic acid derivatives [9, 13, 14] has been reported from the title plant. Diverse biological activities such as antiinflammatory, cytotoxic, antioxidant, antibacterial, antiviral, wound healing, analgesic, acarcidal, and insecticidal activities of the leaf extracts of *E. adenophorum* have been reported [8–10]. In the bioassays of the isolates, cadinene sesquiterpenes and phenolic compounds have been found to be the active principles of *E. adenophorum* [8]. Thus, 9-oxo-10,11-dehydroageraphorone showed an EC_{50} value of 0.325 mg/mL against *Fusarium oxysporum* and 5-*O*-*trans*-*o*-coumaroylquinic acid methyl ester showed an IC_{50} value of 542.3 µM against *Magnaporthe grisea* [8]. Chlorogenic acid methyl ester showed scavenging properties against DPPH radicals with an IC_{50} of 212.2 µM [8]. Thymol derivatives (7,8,9-trihydroxythymol and 7-formyl-9-isobutyryloxy-8-hydroxythymol) showed cytotoxic activity against human cancer cell lines MCF-7, NCI-H460, and HeLa with the respective IC_{50} values ranging from 44.65 to 83.19 µM [8]. 9-Oxo-10,11-dehydroageraphorone, 9-oxoageraphorone, and 9β-hydroxyageraphorone showed insecticidal effects against *Psoroptes cuniculi* [8]. So far, most of the previous studies have focused on the extractives in organic solvents; water-soluble fractions from the leaves of *E. adenophorum* have not been investigated. Most of the reported biological studies have been performed with the extracts, and the bioactive compounds present in the extracts have not been identified. There are not any investigations on the chemical constituents and biological activities of *E. adenophorum* growing in Vietnam.

Acetylcholinesterase (AChE) is an enzyme that degrades the neurotransmitter acetylcholine in the nerve synapse. AChE inhibitors are candidates for screening agents to treat neurodegenerative diseases such as Alzheimer's disease. Some flavonoids isolated from *E. adenophorum* such as quercetagetin-7-*O*-(6-*O*-caffeoyl-β-D-glucopyranoside), 5,4'-dihydroxytlavone, and quercetin-3-*O*-β-D-glucopyranoside showed inhibitory activities against AChE from *Caenorhabditis elegans* (IC_{50} values ranged from 12.54 to 89.06 µg/mL) and *Spodoptera litura* (IC_{50} values ranged from 12.08 to 86.01 µg/mL) [15]. The acyl glucose moiety in the flavonoid glycosides and the number of hydroxyl groups favoured the AChE inhibitory activity, and among the flavonoids, quercetagetin-7-*O*-(6-*O*-caffeoyl-β-D-glucopyranoside) displayed the highest inhibitory effect against AChE with IC_{50} values of 12.54 µg/mL and 12.58 µg/mL, respectively [15].

Effective control of hyperglycemia in type 2 diabetes mellitus includes the inhibition of carbohydrate hydrolysing enzymes such as α-amylase and α-glycosidase with α-glycosidase being the most important enzyme in carbohydrate digestion. Flavonoids are kown as modulators of the enzyme activity, and flavonoids with two catechol groups of the A- and B-rings, together with a 3-OH group of the C-ring present lower IC_{50} values [16]. However, the previous study mainly focused on flavonoid aglycones and did not show the influence of the glycosidic or acyl glycosidic moieties of flavonoid glycosides on inhibitory activity. *E. adenophorum* methanol extract was found to inhibit α-amylase activity with an IC_{50} value of 1.84 ± 0.007 mg/mL [17]. There are no reports on α-glycosidase inhibitory activity of flavonoid constituents from the extract. The objective of this study was to investigate the water-soluble constituents of a MeOH extract from the leaves of *E. adenophorum* growing in Vietnam. α-Glycosidase and acetylcholinesterase inhibition assays were also performed to determine antidiabetic and anti-AChE properties of acylated polyhydroxyflavonoid glycosides found in the water-soluble fraction.

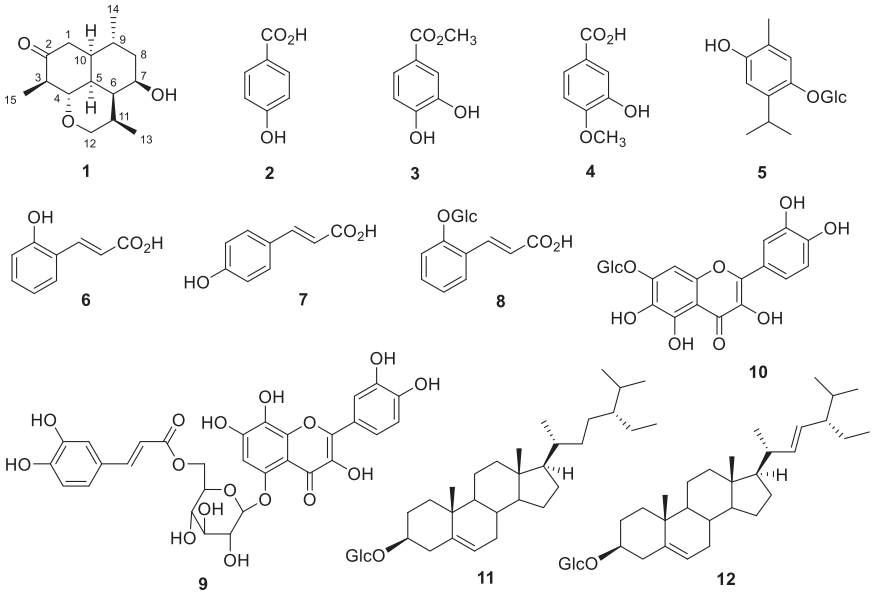

Fig. 1 Chemical structures of compounds **1**–**12**

Results and discussion

Chemistry

The methanolic extract of the dried leaves of *E. adenophorum* was subjected to sequential liquid-liquid fractionation with *n*-hexane and CH_2Cl_2. The water phase was concentrated and separated by a reversed-phase Diaion HP-20 copolymer column. Repeated column chromatography (CC) led to the isolation of phenolic acids (**2**, **3**, **4**, **6**, and **7**), thymol (**5**), phenylpropenoic acid (**8**), flavonols (**9** and **10**), sterol glucosides (**11** and **12**), and a new cadinane sesquiterpenoid, adenophorone (**1**) (Fig. 1). The known compounds from *E. adenophorum* were identified as 4-hydroxybenzoic acid (**2**) [18], methyl protocatechuate (**3**) [18, 19], isovanillic acid (**4**) [18], 5-*O*-glucopyranosylthymoquinol (**5**) [20], *o*- and *p*-coumaric acids (**6** and **7**) [21, 22], 2-*O*-*β*-D-glucopyranosylcinnamic acid (**8**) [23, 24], gossypetin 5-*O*-(6″-(*E*)-caffeoyl)-*β*-D-glucopyranoside (**9**) [25], quercetagetin 7-*O*-*β*-D-glucopyranoside (**10**) [23, 26] by comparison of their spectral data (MS and NMR) with those reported in the literature.

Adenophorone (**1**) was obtained as a white amorphous powder, $[\alpha]_D^{23}$ −33.3 (*c* 0.07, CH_3CN). The molecular formula of **1** was established as $C_{15}H_{24}O_3$ from the HR-ESI-MS peak at *m/z* 275.1621 ($[M+Na]^+$, calcd. 275.1618). The ^1H NMR spectrum (CD_3OD) showed the presence of three methyl doublets at δ_H 0.87 (3H, d, $J = 6.5$ Hz, H-14), 1.01 (3H, d, $J = 7.1$ Hz, H-13), and 1.02 (3H, d, $J = 6.3$ Hz, H-15). In addition, two oxymethines at δ_H 3.88 (1H, d, $J = 10.8$ Hz, 10.6 Hz, H-4) and 4.20 (1H, td, $J = 3.1$ Hz, 2.9 Hz, H-7), and an oxymethylene at δ_H 3.58 (1H, dd, $J = 10.7$ Hz, 5.4 Hz, H-12a) and 3.97 (1H, dd, $J = 11.8$ Hz, 10.7 Hz, H-12b) were observed. Based on HSQC correlations, two methylenes at δ_H 1.26 (1H, ddd, H-8a) and 1.73 (1H, ddd, H-8b), and 2.56 (1H, ddd, H-1a) and 2.60 (1H, dd, H-1b) together with six methines at δ_H 1.53 (1H, m, H-9), 1.68 (1H, m, H-10), 1.71 (1H, m, H-6), 2.07 (1H, m, H-11), 2.08 (1H, dt, H-5), and 2.38 (1H, dqd, H-3) were identified. The ^{13}C NMR spectroscopic data of **1** aided by HSQC correlations confirmed that the molecule contained 15 carbons, including a ketone carbonyl group at δ_C 213.2 (C-2), three methyls at δ_C 13.4 (C-13), 19.8 (C-14), and 10.8 (C-15), two oxymethines at δ_C 68.4 (C-7) and 80.5 (C-4), an oxymethylene at δ_C 72.1 (C-12), two methylenes at δ_C 44.6 (C-1) and 45.3 (C-8), and five methines at δ_C 26.6 (C-9), 35.9 (C-11), 43.1 (C-6), 45.9 (C-5), 46.0 (C-10), and 52.2 (C-3). By comparing the spectroscopic data of **1** with related sesquiterpenoid skeletons having two methyl doublets and an isopropyl group, it was assumed that **1** had the cadinane skeleton. ^1H-^1H COSY, HSQC, and HMBC correlations (Fig. 2) were used to verify the suggestion and to place the functional groups and substituents in the planar structure of **1**. ^1H-^1H COSY correlations showed two spin systems: H_3-15/H-3/H-4/H-5/H-6/H-11(/H-12)/H_3-13 and H-1/H-10/H-9(/H_3-14)/H-8/H-7. The two spin systems were connected through ^1H-^1H COSY interactions between H-5

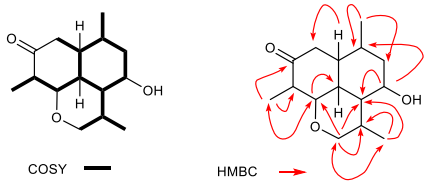

Fig. 2 ^1H-^1H COSY and HMBC correlations of **1**

and H-10 and between H-6 and H-7. The ketone carbonyl group (δ_C 213.2) was placed at C-2 based on HMBC correlations between H-15 and C-2, and H-15 and C-3. An ether oxygen bridge was identified between C-4 and C-12 based on the HMBC correlation between H-12 and C-4. Finally, the 7-hydroxy group was assigned based on HMBC correlations between H-7 and C-6, and H-7 and C-9. Thus, the planar structure of **1** was unambiguously determined as 4,12-epoxy-7-hydroxycadinan-2-one.

The relative stereochemistry of **1** was elucidated based on NOESY correlations (Fig. 3). The same β-orientation of CH_3-15 and H-4 was deduced from NOESY correlations between H-4 and C-15 methyl protons. The opposite α-orientation of CH_3-14 was determined based on the NOESY correlation between H-4β and H-9. The α-orientation of H-10 was determined based on NOESY correlations between CH_3-14 and equatorial H-1a, CH_3-14 and H-8a/H-8b, and CH_3-14 and H-10, as well as H-10 and H-8a. H-5 was placed on the opposite face from that of H-4β and CH_3-15 (α-orientation), since H-3α gave NOE with H-1b and H-1b with H-5. H-6 did not give NOE with CH_3-13 and was assigned to possess an opposite α-orientation. H-7 was assigned α-orientated based on NOESY correlations of CH_3-13 and H-7. The analysis of NOESY spectroscopic data resulted in the structure of **1** as shown in Fig. 1. Configurational analysis for **1** was performed by comparison of calculated and experimental electronic circular dichroism (ECD) data. The experimental ECD spectrum (solid curve) in Fig. 4 (CD curves) showed a close resemblance to the calculated one (dotted curve) for **1**. Therefore, the structure of adenophorone (**1**) was elucidated to be (3*R*,4*S*,5*S*,6*S*,7*R*,9*R*,10*S*,11*R*)-4α,12-epoxy-7β-hydroxycadinan-2-one.

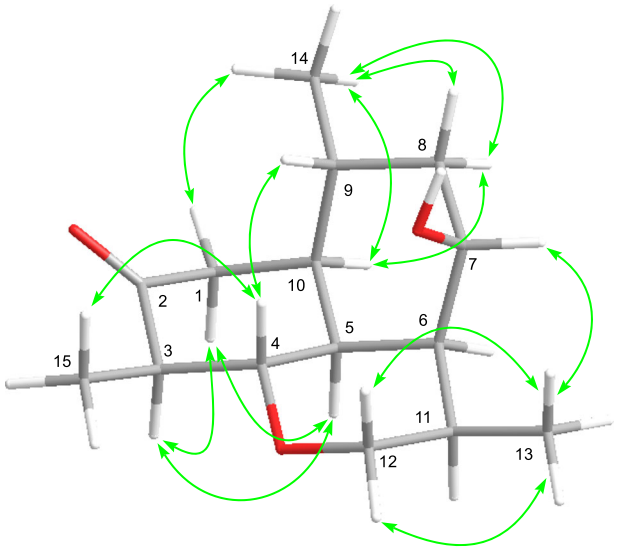

Fig. 3 Phase-sensitive NOESY correlations of **1**

α-Glycosidase and AchE inhibition assays of compounds 9 and 10

α-Glycosidase aids in the digestion of carbohydrates by cleaving complex carbohydrates to yield glucose, since only monosaccharides such as glucose or fructose can be absorbed into the bloodstream. Two membrane-bound intestinal α-glycosidases, maltase-glucoamylase and sucrase-isomaltase, cleave α-(1,4)-glycosidic linkages to release α-D-glucose in the human digestive system. Inhibiting the function of these enzymes in patients with type 2 diabetes reduces hyperglycemia (high blood sugar). Hyperglycemia mainly affects people with diabetes and can become serious if not treated. α-Glycosidase inhibitors (AGI), such as acarbose and miglitol, are FDA-approved drugs that treat type-2 diabetes [27]. AGIs lower the effect of postprandial sugar in the short term by blocking the breakdown of starchy foods and slowing down the absorption of some sugars. In vitro, the α-glycosidase inhibition assay substrate, *p*-nitrophenyl α-D-glucopyranoside (pNPG), is hydrolyzed by α-glycosidase to *p*-nitrophenyl and its absorbance at 410 nm is measured. The colorimetric

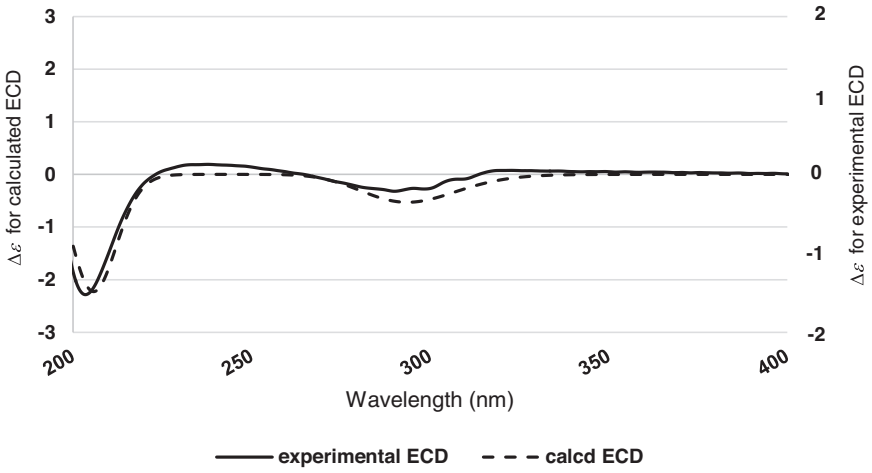

Fig. 4 Experimental and calculated ECD spectra of **1**

quantification was used to calculate α-glycosidase inhibitory activity (%) and half-maximal inhibitory concentration (IC_{50}). Table 1 shows the results of α-glycosidase inhibitory activity of compounds **9** and **10**. At 256 µg/mL, gossypetin 5-*O*-(6″-(*E*)-caffeoyl)-β-D-glucopyranoside (**9**) inhibited α-glycosidase activity by 80%. An IC_{50} of 24.0 ± 1.61 µg/mL was determined, while acarbose showed an inhibitory activity of 134.56 ± 3.02 µg/mL as a positive control. At 256 µg/mL, quercetagetin 7-*O*-β-D-glucopyranoside (**10**) inhibited α-glycosidase inhibitory activity by only 27%. According to [16], the aglycones of compounds **9** and **10** satisfy the structural requirements for the α-glycosidase inhibitory activity, including the catechol moiety in the B-ring and 3-OH in the C-ring, however, the loss of 5-OH or 7-OH may reduce the activity. The result of the inhibition assay of **10** reflects the loss of 7-OH since quercetagetin showed the α-glycosidase inhibitory activity with an IC_{50} value of 180.11 ± 3.68 µM [28]. Regarding the potent activity of compound **9**, additional caffeoyl hydroxy groups in the glucose moiety may be involved in binding to the active site of the enzyme.

Acetylcholine (ACh) is a neurotransmitter associated with cognitive, autonomic, and neuromuscular functions. ACh is synthesized from choline and acetyl CoA with the enzymatic catalysis of choline acetyltransferase. ACh has a very short lifetime; it is broken down by the enzyme acetylcholinesterase (AChE) to choline and acetic acid, and thus the neurotransmission process is terminated. Choline is then reentered into nerve cells for the synthesis of ACh. There are lower levels of ACh in the brain of a person with Alzheimer's disease, harming the communication between nerve cells, and some of the nerve cells that use ACh are also lost. Chemicals that inhibit AChE activity interfere with the hydrolysis process, leading to the accumulation of ACh and a prolonged lifetime of ACh at nerve synapses, and hence easing the symptoms of Alzheimer's disease. Some natural alkaloids, such as galantamine (galanthamine) from *Galanthus*, *Narcissus*, and *Leucojum* species of the Amaryllidaceae family and huperzine A from *Huperzia* sp., are available drugs to treat Alzheimer's disease based on the inhibition of AChE. In the AChE inhibition assay in vitro (Ellman's method), the substrate acetylthiocholine iodide (ATCI) is hydrolyzed by AChE into thiocholine, which reacts with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) to form a yellow product, 5-thio-2-nitrobenzoate (TNB). The concentration of TNB is quantified by its absorbance at 412 nm, which reflects AChE activity. The results of the AChE inhibitory activity of compounds **9** and **10** are shown in Table 2. Gossypetin 5-*O*-(6″-(*E*)-caffeoyl)-β-D-glucopyranoside (**9**) inhibited 54% AChE activity at the maximal concentration of 256 µg/mL. The half-maximal inhibitory concentration (IC_{50}) of **9** was determined to be 217.60 ± 15.47 µg/mL; in the same assay, the IC_{50} of donepezil was 0.018 ± 0.008 µg/mL. Quercetagetin 7-*O*-β-D-glucopyranoside (**10**) inhibited only 34% AChE activity at 256 µg/mL. In comparison with the AChE inhibitory activity of quercetagetin-7-*O*-(6-*O*-caffeoyl-β-D-glucopyranoside) [15] the loss of the caffeoyl hydroxy groups in the glucose moiety of compound **10** reduced the activity. Due to the additional binding caffeoyl hydroxy groups in compound **9**, the AChE activity of **9**, albeit weak, may be explained.

Table 1 Results of α-glycosidase inhibitory activity of compounds **9** and **10**

Compound	α-Glycosidase inhibitory activity (%)					IC_{50} (µg/mL)
	256 µg/mL	64 µg/mL	16 µg/mL	4 µg/mL	1 µg/mL	
9	80	67.5	46.5	40	13	24.0 ± 1.61
10	27	24	18	11	0	>256
Acarbose: IC_{50} 134.56 ± 3.02 µg/mL						

Materials and Methods

General

Optical rotations were measured on a Jasco P-2200 digital polarimeter. IR spectra were measured on a JASCO FT/IR-6100 spectrophotometer. The UV and ECD spectra were obtained with a JASCO J-1100 spectropolarimeter. ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, HMBC, and NOESY spectra were obtained on a Bruker Avance 500 MHz NMR spectrometer (Billerica, MA, USA) at 500 MHz and 125 MHz or on a Bruker Avance III spectrometer at 600 MHz and 150 MHz. CD_3OD and DMSO-d_6 were NMR solvents, and TMS (tetramethylsilane) was used as the reference standard. ESI-MS spectra were measured on an Agilent LC-MS Ion Trap LC/MS system (Santa Clara, CA, USA). HR-ESI-MS

Table 2 Results of AChE inhibitory activity of compounds **9** and **10**

Compound	AChE inhibitory activity (%)					IC_{50} (µg/mL)
	256 µg/mL	64 µg/mL	16 µg/mL	4 µg/mL	1 µg/mL	
9	54	34	0	0	0	217.60 ± 15.47
10	34	21	0	0	0	>256
Donepezil: IC_{50} 0.018 ± 0.008 µg/mL						

spectra were measured on a Thermo Fisher Scientific LTQ Orbitrap XL mass spectrometer (Whatham, MA, USA). Thin-layer chromatography (TLC) was performed on Merck pre-coated TLC silica gel 60 F_{254} sheets (Darmstadt, Germany), and the spots were detected by spraying with 1% vanillin (China)-concentrated H_2SO_4 (China) or 5% $FeCl_3$ (China)-EtOH (China) and then heating on a hot plate. Column chromatography (CC) was performed on Merck silica gel of 63–200 µm, 40–63 µm, or 15–40 µm (Darmstadt, Germany) particle size and reversed-phase Diaion HP-20 (Mitsubishi Chemicals, Japan). Solvents for column chromatography: *n*-hexane (Korea), dichloromethane (Taiwan), acetone (Taiwan), ethyl acetate (Singapore), and MeOH (Indonesia), were distilled and dried over Na_2SO_4 (China) before use.

Plant material

The leaves of *E. adenophorum* were collected in September 2016 in Sapa, Lao Cai Province, Vietnam. The leaves were shade-dried and the oven-dried at 40 °C. The plant was identified by Dr. Nguyen Thi Kim Thanh (Faculty of Biology, VNU University of Science, VNU Hanoi). A voucher specimen (EA-09-16) was deposited at the same institute.

Extraction and isolation

The dried leaves of *E. adenophorum* (1.34 kg) were powdered and extracted three times with MeOH (each time for 7 days) at room temperature. The combined MeOH extract was evaporated using a Büchi RType (KRvr 65/45) evaporator under reduced pressure to yield a MeOH extract. The MeOH extract was partitioned between H_2O and *n*-hexane and then dichloromethane successively to afford the corresponding soluble fractions. The water phase was concentrated and separated by using a Diaion HP-20 column (6 cm i.d. × 100 cm length) with a MeOH-H_2O solvent gradient (20, 40, 60, and 100%, v/v) to yield four corresponding fractions. The 60% fraction (13.1 g) was chromatographed on a silica gel (63–200 µm) column (3.0 cm i.d. × 30 cm length) and eluted with a stepwise gradient of dichloromethane-MeOH (9:1, 6:1, 1:1, v/v); acetone-MeOH (6:1, 3:1, 1:1, v/v) to yield 9 fractions (F1-F9) on the basis of TLC analysis. Fraction F2 was fractionated on silica gel (40–63 µm) with *n*-hexane-ethyl acetate (3:1, 1:1, 1:3, v/v) to yield compounds **1** (2.0 mg), **2** (27.5 mg), **3** (3.0 mg), and **4** (17.3 mg). Fraction F4 was separated on a silica gel (40–63 µm) column eluted with *n*-hexane-acetone (50:1, 12:1, 9:1, 6:1, 3:1, 1:1, v/v) to yield compounds **4** (3.1 mg) and **5** (258.7 mg). Fraction F5 was separated by silica gel (40-63 µm) CC with dichloromethane-ethyl acetate (1:9, 1:15, v/v); ethyl acetate-acetone (6:1, 3:1, 1:1, v/v) to give a mixture of compounds **6** and **7** (5.0 mg). Fraction F6 was separated by silica gel (40–63 µm) CC with ethyl acetate-acetone (9:1, 6:1, 3:1, 1:1, v/v) to yield compound **8** (10.0 mg). Fraction F7 was separated by silica gel (40–63 µm) CC with ethyl acetate-acetone (15:1, 9:1, 6:1, 3:1, 1:1, v/v) to yield compound **9** (51.0 mg). Fraction F8 was recrystallized in MeOH to yield compound **10** (11.7 mg). The 100% fraction (4.55 g) was fractionated on a silica gel (40–63 µm) column (3.0 cm i.d. × 30 cm length) and eluted with a dichloromethane-methanol solvent gradient (9:1, 6:1, 3:1, 1:1, v/v) to yield 9 fractions F1-F9. Fraction F6 was separated by silica gel (40–63 µm) CC with dichloromethane-ethyl acetate (1:3, 1:5, v/v) to yield a mixture of compounds **11** and **12** (3.0 mg).

Adenophorone (1)

White amorphous powder. $[\alpha]_D^{23}$ −33.3 (*c* 0.07, CH_3CN). UV λ_{max} (CH_3CN) log ε (nm): 2.56 (205), 1.11 (286). IR (KBr) ν_{max} cm^{-1}: 3456, 2946, 2899, 1706, 1458, 1372, 1069, 1034. ECD $\Delta\varepsilon$ (nm): −1.52 (204), −0.21 (290) (*c* 3.97×10^{-3} M, CH_3CN). Positive-ion HR-ESI-MS: 275.1621 (calculated for $C_{15}H_{24}O_3Na$: 275.1618). ^{1}H-NMR (CD_3OD): δ 0.87 (3H, d, J = 6.5 Hz, H-14), 1.01 (3H, d, J = 7.1 Hz, H-13), 1.02 (3H, d, J = 6.3 Hz, H-15), 1.26 (1H, ddd, J = 13.9 Hz, 12.0 Hz, 2.9 Hz, H-8a), 1.53 (1H, m, H-9), 1.68 (1H, m, H-10), 1.71 (1H, m, H-6), 1.73 (1H, ddd, J = 13.9 Hz, 3.2 Hz, 3.1 Hz, H-8b), 2.07 (1H, m, H-11), 2.08 (1H, dt, J = 10.8 Hz, 4.8 Hz, H-5), 2.38 (1H, dqd, J = 10.6 Hz, 6.3 Hz, 0.9 Hz, H-3), 2.56 (1H, ddd, J = 13.4 Hz, 5.9 Hz, 0.9 Hz, H-1a), 2.60 (1H, dd, J = 13.4 Hz, 2.8 Hz, H-1b), 3.58 (1H, dd, J = 10.7 Hz, 5.4 Hz, H-12a), 3.88 (1H, dd, J = 10.8 Hz, 10.6 Hz, H-4), 3.97 (1H, dd, J = 11.8 Hz, 10.7 Hz, H-12b), 4.20 (1H, td, J = 3.1 Hz, 2.9 Hz, H-7). ^{13}C-NMR (CD_3OD): δ 10.8 (C-15), 13.4 (C-13), 19.8 (C-14), 26.6 (C-9), 35.9 (C-11), 43.1 (C-6), 44.6 (C-1), 45.3 (C-8), 45.9 (C-5), 46.0 (C-10), 52.2 (C-3), 68.4 (C-7), 72.1 (C-12), 80.5 (C-4), 213.2 (C-2).

Theoretical ECD calculation for the determination of absolute configuration of 1

Configurational analysis was performed with the Spartan′20 V1.1.2. program (Wavefunction, Inc., Irvine, CA, USA) on a commercially available personal computer [operating system: Microsoft 64-bit version of Windows 10 Home edition; 16-core central processing unit: Ryzen 9 5950X processor (Advanced Micro Devices, Inc., Santa Clara, CA, U.S.A.) run at 3.4 GHz; random access memory: 32 GB]. Stable conformers up to 40 kcal/mol for **1** were initially searched using the Merck molecular force field method. Then the aforementioned stable conformers were further optimized using the Hartree-Fock (HF)/3-21 G and ωB97XD/6-31 G* programs. The resulting conformers were subjected to ECD calculations, and the ECD calculations for these conformers were performed with Gaussian 16 (Revision A.03 by Gaussian) [29] on the ChemPark cloud system

[30]. The dominant conformers of **1** capable of covering >99% of the population according to Boltzmann's distribution were selected. Time-dependent density functional theory calculations were conducted at the CAM-B3LYP/TZVP level for these conformers. The resulting rotational strength data were converted to Gaussian curves (bandwidth sigma = 0.6 eV) to obtain the ECD spectra of each conformer, and the spectra were combined after Boltzmann weighting according to their population contributions. The wavelength of the spectra was corrected (+15 nm) based on the absorptions of about 200–250 nm (referring to the experimental and calculated UV spectra) to give the corresponding theoretical ECD spectrum.

α-Glycosidase inhibition assay

The inhibition of α-glycosidase activity was carried out following the method described [31]. A 2.5 mM solution of *p*-nitrophenyl α-D-glucopyranoside (*p*-NPG) (Sigma-Aldrich) and 0.2 U/mL α-glycosidase from *Saccharomyces cerevisiae* (Sigma-Aldrich) were prepared in 100 mM potassium phosphate buffer at pH 6.8. The sample (compound **9** or **10**) was prepared in dimethylsulfoxide (DMSO) (Sigma-Aldrich) and serially diluted in the concentrations of 1, 4, 16, 64, and 256 µg/mL. 10 µL of sample was added to a reaction mixture consisting of 40 µL of 100 mM phosphate buffer at pH 6.8, 25 µL of 0.2 U/mL α-glycosidase in a 96-well microplate, and the reaction mixture was incubated for 10 min. at 37 °C. Then, 25 µL of 2.5 mM *p*-NPG was added, and the reaction mixture was further incubated for 20 min. at 37 °C. After 30 min. 100 mM sodium carbonate solution (100 µL) was added to stop the reaction. The absorbance of the mixture was measured at λ 410 nm on a UV-VIS spectrophotometer (Biotek Instruments, USA). To make a control reaction, the tested sample was replaced by 10 µL of 100 mM phosphate buffer at pH 6.8. Acarbose (Sigma-Aldrich) was used as the reference standard. The α-glycosidase inhibitory activity was calculated using the following equation: α-glycosidase inhibitory activity $(\%) = (A_{control} - A_{sample}) / A_{control} \times 100$, where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample. IC_{50} (half-maximal inhibitory concentration) [32] was calculated using Tablecurve software. The experiments were repeated in triplicate. The data were expressed as means of three replicate determinations ± standard error of the mean (SEM).

Acetylcholinesterase inhibition assay

The inhibition of acetylcholinesterase (AChE) activity was carried out following the colorimetric method previously described [33–35]. The experiment was performed in a 96-well microplate. The sample (compound **9** or **10**) and AChE solution (0.25 U/mL) (Sigma-Aldrich) were added to each well, and the solution was mixed and incubated for 15 min. at 25 °C. Then solutions of 2.4 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) reagent (Sigma-Aldrich) and acetylcholine iodide (ATCI) substrate (Sigma-Aldrich) were further incubated for 15 min. at 25 °C. The absorbance of the mixture was measured at λ 410 nm on a UV-VIS spectrophotometer (Biotek Instruments, USA). Donepezil (Sigma-Aldrich) was used as the positive reference standard. The AChE inhibitory activity was calculated using the following equation: AChE inhibitory activity $(\%) = (A_{control} - A_{sample}) / A_{control} \times 100$, where $A_{control}$ is the absorbance of the control, A_{sample} is the absorbance of the sample. IC_{50} (half-maximal inhibitory concentration) [32] was calculated using Tablecurve software. The experiments were repeated in triplicate. The data were expressed as means of three replicate determinations ± standard error of the mean (SEM).

Conclusions

We described the first study of the chemical constituents of the water-soluble fraction from the leaves of *E. adenophorum*. Twelve compounds were isolated, including a new cadinane sesquiterpenoid, three simple phenolic acids, a thymol derivative, three phenylpropenoic acids, two sterol glucosides, and two polyhydroxyflavonol glycosides. The absolute configuration of the new cadinane sesquiterpenoid was determined by theoretical ECD calculation; and this is also the first example of the determination of the absolute configuration of sesquiterpenoids in *E. adenophorum*. Two polyhydroxyflavonol glycosides are the rare naturally occurring flavonoids. In the α-glycosidase and acetylcholinesterase inhibition assays, gossypetin 5-*O*-(6″-(*E*)-caffeoyl)-β-D-glucopyranoside exhibited IC_{50} values of 24.0 ± 1.61 µg/mL and 217.60 ± 15.47 µg/mL, respectively, while quercetagetin 7-*O*-β-D-glucopyranoside was slightly active. The results are in agreement with the bioactivity tendency of flavonoid glycosides, where the acylated glucopyranosyl moiety is favourable for their inhibitory effects. Since the synergistic properties of extracts from medicinal plants depend on the presence of nonactive and active phytochemicals, the disclosure of the chemical profiles of the water-soluble fraction lays the basis for the possible development of therapeutic herbal formulations from *E. adenophorum*.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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