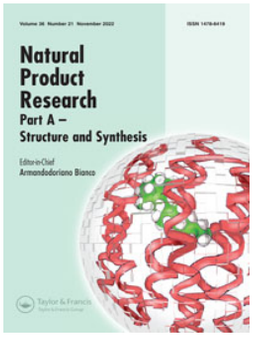

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Phytochemical constituents from *Elsholtzia ciliata* (Thunb.) Hyl. and their nitric oxide production inhibitory activities

Duong Thi Dung[a,b], Nguyen Huy Hoang[a,b], Duong Thi Hai Yen[a], Pham Hai Yen[a], Vu Kim Thu[c], Nguyen Viet Dung[a], Ngo Anh Bang[a], Do Thi Trang[a], Nguyen Thi Cuc[a], Huong Phan Thi Thanh[a], Bui Huu Tai[a,b], Nguyen Xuan Nhiem[a,b] and Phan Van Kiem[a,b]

[a]Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam; [b]Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam; [c]Faculty of Basic Sciences, Hanoi University of Mining and Geology, Ha Noi, Vietnam

ABSTRACT

A new megastigmane glycoside, (3*S*,4*R*,7*E*)-megastigma-5,7-diene-9-one-3,4-diol 3-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside (**1**) and a new cyanogenic glycosyl derivative, (*S*)-2-(6′-*O*-*R*-rosmarinoyl-*β*-D-glucopyranosyloxy)-phenylacetonitrile (**2**) were isolated from the methanol extract of the *Elsholtzia ciliata* together with twelve known compounds, 1-*O*-*β*-D-glucopyranosyl-2-hydroxy-4-allylbenzene (**3**), citrusin C (**4**), 1,2-di-*O*-*β*-D-glucopyranosyl-4-allylbenzene (**5**), manglieside B (**6**), 4-allyl-2-hydroxyphenyl 1-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**7**), (-)-isolariciresinol 3*α*-*β*-D-glucopyranoside (**8**), 7*R*,8*R*-threo-4,7,9-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-9′-*O*-*β*-D-glucopyranoside (**9**), 7*R*,8*R*-threo-4,7,9,9′-tetrahydroxy-3-methoxy-8-*O*-4′-neolignan-9′-*O*-*β*-D-glucopyranoside (**10**), cedrusin-4-*O*-*β*-D-glucopyranoside (**11**), icariside E_3 (**12**), everlastoside L (**13**) and rosmarinic acid (**14**). Their chemical structures were elucidated on the basic of extensive 1D and 2D-NMR experiments, as well as their mass spectroscopic data. The absolute configurations of the compounds **1** and **2** were successfully indicated by both theoretical and calculated CD spectra. Compounds **3**–**7**, **9** and **10** potential inhibited NO production in LPS-activated RAW264.7 cells with IC_{50} values of 6.71, 8.97, 12.38, 14.27, 16.13, 13.54, 16.27 μM, respectively, compared to that of the positive control of N^G-monomethyl-L-arginine acetate (L-NMMA), IC_{50} = 32.51 μM.

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CONTACT Phan Van Kiem phankiem@yahoo.com; CONTACT Pham Hai Yen yeninpc@yahoo.com

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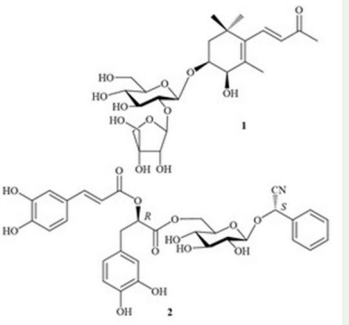

List of abbreviations: HRESIMS: high-resolution electrospray ionization mass spectrometry; NMR: Nuclear Magnetic Resonance; HMBC: Heteronuclear Multiple Bond Correlation; HSQC: Heteronuclear Single Quantum Coherence; COSY: Correlation Spectroscopy; NOESY: Nuclear Overhauser Effect Spectroscopy; NO: Nitric oxide; LPS: Lipopolysaccharide; L-NMMA: N^G-monomethyl-L-arginine acetate salt; Prep-HPLC: Preparative High Performance Liquid Chromatography; CD: Circular Dichroism Spectroscopy

1. Introduction

The *Elsholtzia* genus (Lamiaceae family) comprises 44 species, which are widely distributed in East Asia, Africa, North America, and European. The most common uses of *Elsholtzia* species are for the treatment of cold, fever, dysentery, diarrhoea, heat stroke, digestion disorder, and detoxication. A lot of *Elsholtzia* species are used as remedies for anti-inflammation and analgesic, such as *E. blanda*, *E. bodinieri*, *E. densa*, *E. fruticosa*, *E. rugulosa*, *E. penduliflora*, *E. stauntoni*, *E. myosurus*, and *E. ciliate* (Liu et al. 2007). The previous investigations have reported that the main chemical composition of *E. ciliata* are volatile oil, steroids, flavonoids, and triterpenoids (Zhang et al. 2021), phenolic glucosides and flavone glycosides (Nugroho et al. 2019; Pudziuvelyte et al. 2020; Seo et al. 2020). Some of these components showed anti-inflammatory, cytotoxic, analgesic, and antioxidant activities. In the Vietnamese folk medicines, the aerial parts of *E. ciliata* has been used for treating dermatitis, haemorrhoids, and arthritis (Bich et al. 2004). To clarify more about the bioactive substances of this plant, the aerial parts of *E. ciliate* was phytochemical investigated. This paper reports the isolation of two new (**1–2**) and twelve known compounds (**3**–**14**) together with their inhibitory activity on NO production in LPS-activated RAW264.7 cells. Their chemical structures of the isolated compounds were elucidated on the basis of spectroscopic analyses (IR, UV, HRESIMS, and 1D and 2D NMR, CD) and the TDDFT ECD calculations in comparison with the previous reported data.

2. Results and discussion

Fourteen compounds were isolated from the aerial parts of *E. ciliate* by the phytochemical investigation. Of these, the known compounds were identified to be 1-*O*-

β-D-glucopyranosyl-2-hydroxy-4-allylbenzene (**3**) (Ly et al. 2002), citrusin C (**4**) (Kim et al. 2004), 1,2-di-*O*-β-D-glucopyranosyl-4-allylbenzene (**5**) (Ly et al. 2002), manglieside B (**6**) (Kiem et al. 2008), 4-allyl-2-hydroxyphenyl 1-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**7**) (Yamauchi et al. 2007; (-)-isolariciresinol 3α-β-D-glucopyranoside (**8**) (Achenbach et al. 1992), 7*R*,8*R*-threo-4,7,9-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-9′-*O*-β-D-glucopyranoside (**9**) (Matsuda and Kikuchi 1996), 7*R*,8*R*-threo-4,7,9,9′-tetrahydroxy-3-methoxy-8-*O*-4′-neolignan-9′-*O*-β-D-glucopyranoside (**10**) (Matsuda and Kikuchi 1996), cedrusin-4-*O*-β-D-glucopyranoside (**11**) (Agrawal et al. 1983), icariside E_3 (**12**) (Lee et al. 2001), everlastoside L (**13**) (Morikawa et al. 2009) and rosmarinic acid (**14**) (García et al. 2016) by the consistent of their spectral data, including CD, with those reported in the literature.

The molecular formula of compound **1** (Figure 1) was determined as $C_{24}H_{38}O_{12}$ by the HRESIMS ion peaks at *m/z* 553.2030 $[M + Cl]^-$ (calcd. for $[C_{24}H_{38}O_{12}\ Cl]^-$, 553.2057) and *m/z* 517.2252 $[M - H]^-$ (calcd. for $[C_{24}H_{37}O_{12}]^-$, 517.2291). The ^1H NMR spectrum of

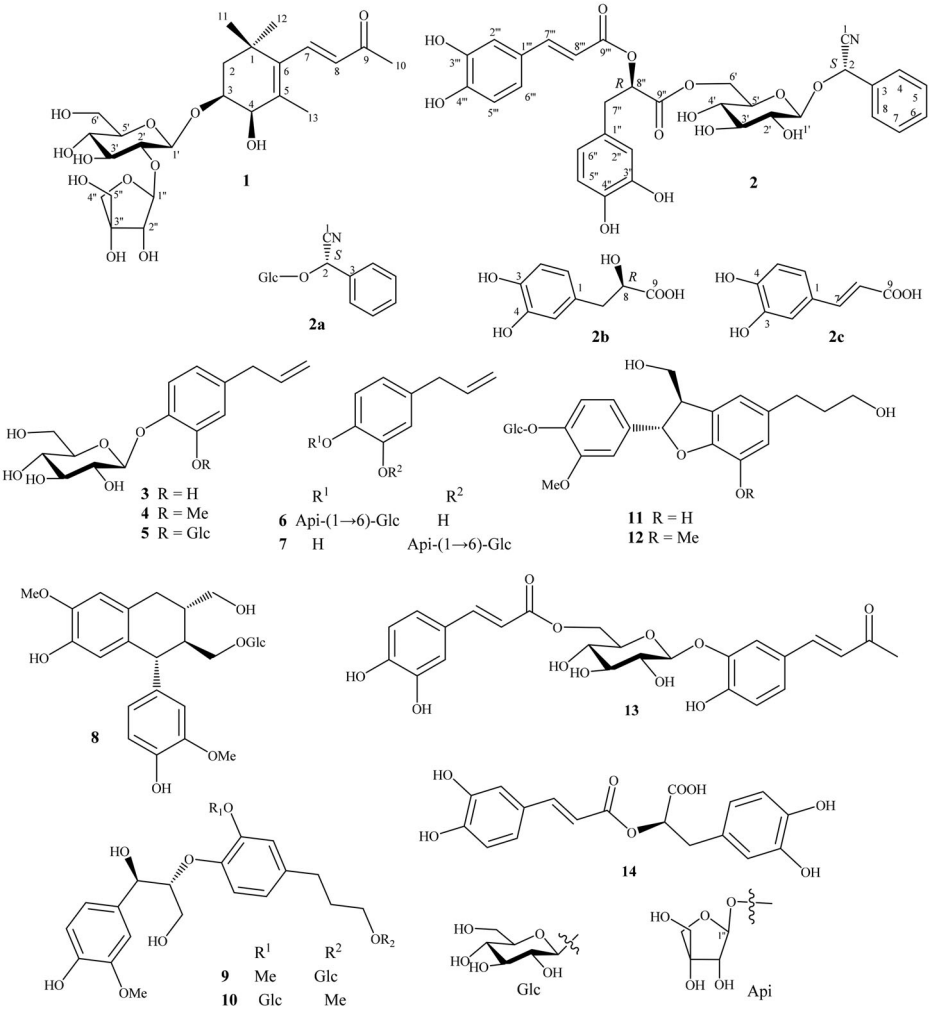

Figure 1. Chemical structures of compounds **1–14** from *Elsholtzia ciliata*.

1 showed signals of four quaternary methyls [δ_H 1.12 (s, H-11), 1.16 (s, H-12), 1.90 (s, H-13) and 2.34 (s, H-10)], one *trans* double bond [δ_H 6.16 (d, $J = 16.2$ Hz, H-7) and δ_H 7.30 (d, $J = 16.2$ Hz, H-8)], two oxygenated methine groups [δ_H 4.07 (ddd, $J = 12.6$, 3.6, 3.6 Hz, H-3) and δ_H 4.15 (d, $J = 3.6$ Hz, H-4)], one methylene group [δ_H 1.64 (ddd, $J = 12.6$, 3.6, 1.2 Hz, H-2*eq*) and δ_H 1.96 (dd, $J = 12.6$, 12.6 Hz, H-2$_{ax}$)]. The appearance of the two anomeric protons at δ_H 4.60 (d, $J = 7.8$ Hz) and δ_H 5.26 (d, $J = 3.6$ Hz) suggested two sugar moieties. The ^{13}C NMR and HSQC spectra of **1** indicated 24 carbons, including 13 carbons of the megastigman aglycon and 11 carbons of the glucose and apiose skeletons (Table S1). Four methyls [δ_C 27.2 (C-10), 30.3 (C-11), 28.0 (C-12), and 20.0 (C-13)], one ketone [δ_C 200.9 (C-9)], two double bonds [δ_C 132.5 (C-5), 141.2 (C-6), 144.3 (C-7), and 134.7 (C-8)], and two oxygenated methine carbons [δ_C 75.4 (C-3) and 69.8 (C-4)] were assigned for the aglycone part (Yu et al. 2005; Lee et al. 2011; Yan et al. 2017), and the sets of signals [δ_C 101.6, 83.6, 77.8 × 2, 71.5 (5CH), and 62.6 (CH_2)] and [δ_C 112.4 (CH), 77.6 (CH), 79.7 (C), 74.8 (CH_2) and 65.0 (CH_2)] were suggested for a glucose and an apiose moiety, respectively (Pawar and Bhutani 2004). The aglycon NMR data of **1** (Table S1) were very similar to those of komaroveside A (Lee et al. 2011) measured in the same solvent (CD_3OD) suggesting the megastigma-5,7-diene-9-one-3,4-diol skeleton, which was further evidenced by HMBC correlations as shown in Figure S1. The large value, $J_{H\text{-}7/H\text{-}8} = 16.2$ Hz, indicated the *trans*-configuration of the C-7/C-8 double bond. In the NOESY spectrum of **1** (Figure S2), the cross peaks of H_3-12 (δ_H 1.16) and H-3$_{ax}$ (δ_H 4.07), H_3-11 (δ_H 1.12) and H-2$_{ax}$ (δ_H 1.96), H_3-12 (δ_H 1.16) and H-2$_{eq}$ (δ_H 1.63) were observed indicating that H-12, H-2$_{eq}$, and H-3$_{ax}$ were in one side and H-11 and H-2$_{ax}$ were in the other side of the molecule. The small value of the proton coupling constant of H-4 and H-5 ($J = 3.6$ Hz) indicated they are in *cis*-configuration (Yu et al. 2005; Lee et al. 2011), which differed from the *trans*-configuration ($J = 7.8$ Hz) (Yan et al. 2017). In the HSQC spectrum, peaks at δ_H 4.60 (H-1′), 3.32 (H-2′), 3.50 (H-3′), 3.36 (H-4′), and 3.30 (H-5′) corresponding correlated to peaks at δ_C 101.6, 83.6, 77.8, 71.5, 77.8 (C-1′ to C-5′), while peak at δ_C 62.6 correlated to two protons at δ_H 3.69 and 3.88; peaks at δ_H 5.26 and 3.98 correlated with carbons at δ_C 112.4 and 77.6, respectively, while two carbons at δ_C 74.8 and 65.0 correlated with protons at δ 3.77/4.15 and 3.60, respectively. Furthermore, the COSY cross peaks of H-1′/H-2′/H-3′/H-4′/H-5′/H-6′ and H-1″/H-2″ and the HMBC correlations from H-1″ (δ_H 5.26) to C-3′ (δ_C 83.6) and from H-1′ (δ_H 4.60) to C-3 (δ_C 75.4) were observed. These evidence confirmed the apiofuranosyl-(1→2)-glucopyranoside skeleton, which attached to C-3 of the aglycon by an ether linkage. In addition, the carbon chemical shifts, multiplicity and the proton coupling constants of the anomeric signals consistent with the β-glucopyranosyl ($J = 7.5$ Hz) and β-apiofuranosyl ($J = 2.0$ Hz) linkages. The absolute configuration of **1** was elucidated by analysis of its ECD spectrum, ^{13}C NMR spectral data, and acid hydrolysis. The ECD spectrum of **1** showed positive Cotton effects at 274 nm (+1.50 mdeg) and 314 nm (-0.53 mdeg) indicating 3*S*,4*R*-configuration as previously reported (Son et al., 2015). Finally, the presence of D-glucose and D-apiose in **1** was confirmed by acid hydrolysis, HPLC analysis of the products after treatment with cysteine methyl ester and *O*-tolyl isothiocyanate and comparison with those of authentic sugars (Tanaka et al. 2007; Tai et al. 2019). Thus, compound **1** was determined to be (3*S*,4*R*,7*E*)-megastigma-5,7-diene-9-one-3,4-diol 3-*O*-β-D-apiofuranosyl-

(1→2)-β-D-glucopyranoside, a new compound and named elsholciliata A (Figures S3–S14).

Compound **2** was obtained as colourless amorphous powder. The HRESIMS spectrum of **2** showed the quasi molecular ion peaks at *m/z* 672.1484 $[M+Cl]^-$ (calcd. for $[C_{32}H_{31}NO_{13}Cl]^-$, 672.1484) (negative ion mode) and *m/z* 660.1682 $[M+Na]^+$ (calcd. for $[C_{32}H_{31}NO_{13}Na]^+$, 660.1693) (positive ion mode), indicating the molecular formula of $C_{32}H_{31}NO_{13}$. The ^{1}H NMR spectrum of **1** exhibited a mono-substituted aromatic ring (δ_H 7.44-7.58, 5H), six aromatic protons of the two ABX coupled system {[δ_H 7.05 (d, $J=2.0$ Hz, H-2‴), 6.77 (d, $J=8.0$ Hz, H-5‴), 6.95 (dd, $J=8.0$, 2.0 Hz, H-6‴)] and [δ_H 6.79 (d, $J=2.0$ Hz, H-2″), 6.74 (d, $J=8.0$ Hz, H-5″), 6.65 (dd, $J=8.0$, 2.0 Hz, H-6″)]}, a *trans* double bond (δ_H 7.60 and 6.32, each, d, $J=16.0$ Hz), and an anomeric proton at δ_H 4.30 (d, $J=7.5$ Hz). The ^{13}C NMR and HSQC spectra of **1** revealed 32 C including 18 C of three aromatic rings, one double bond, one glucose sugar, and two carbonyl carbons (δ_C 168.5 and 171.6) (Table S2). The caffeoyl moiety was confirmed by HMBC correlations from H-7‴ (δ_H 7.60) to C-1‴ (δ_C 127.6)/C-2‴ (δ_C 115.3)/C-6‴ (δ_C 123.3)/C-9‴ (δ_C 168.5) and from H-8‴ (δ_H 6.32) to C-1‴/C-7‴/C-9‴. The salvianic acid moiety was indicated by COSY correlation between H-7″ (δ_H 3.13) and H-8″ (δ_H 5.30) as well as by HMBC correlations from H-7″ to C-1″ (δ_C 128.9)/C-2″ (δ_C 117.7)/C-6″ (δ_C 121.9)/C-9″ (δ_C 171.6) and from H-8″ (δ_H 5.30) to C-1″/C-7″/C-9″. Furthermore, the HMBC correlation from H-8″ to C-9‴ evidenced an ester linkage from C-8″ and C-9‴ to form the rosmarinoyl moiety. The remaining NMR signals were assigned for a prunasin moiety, including a glucose (δ_C 102.4, 74.7, 77.7, 71.6, 75.8, 65.3) and the mandelonitrile unit (Cardona et al. 1992). The HMBC correlation from H-1′ (δ_H 4.30) to C-2 (δ_C 68.8) and from H-6′ (δ_H 4.31/4.55) to C-9″ (δ_C 171.6) confirmed that the glucose moiety linked to C-2 by an ether linkage and the rosmarinic acid linked to C-6′ of the glucose by an ester linkage. The large $J_{H\text{-}1'/H\text{-}2'}$ value (7.5 Hz) suggested the β-glucoside linkage. Alkaline hydrolysis of **2** obtained prunasin (**2a**) (Cardona et al. 1992), salvianic acid (**2b**), and caffeic acid (**2c**) (Dai et al. 2010), which were identified by their physicochemical data including ^{1}H NMR and CD spectra (Figures S27-S31). The absolute configuration of compounds **2a** and **2b** were determined to be *S* and *R*, respectively (as in *S*-prunasin and *R*-rosmarinic acid) based on the consistent of their experimental ECD spectra with the corresponding theorical TD-DFT calculation ECD of **2a**-*S* and **2b**-*R* isomers (Figures S30 and S31). In addition, the presence of D-glucose in **2** was confirmed by acid hydrolysis, HPLC analysis of the products after treatment with cysteine methyl ester and *O*-tolyl isothiocyanate and comparison with those of authentic sugars (Tanaka et al. 2007; Tai et al. 2019). From the above evidence, compound **2** was elucidated as (*S*)-2-(6′-*O*-*R*-rosmarinoyl-β-D-glucopyranosyloxy)-phenylacetonitrile, a new compound and named elsholciliata B (Figures S15–S26).

As the previous reports, phenolic compounds exhibited potential anti-inflammatory activity (Ambriz-Pérez et al. 2016; Trang et al. 2022). Therefore, compounds **1**–**14** were evaluated for anti-inflammatory activity by their ability to inhibit NO production in LPS stimulated RAW 264.7 cells (Supporting information). All the isolates did not show cytotoxic activity by MTT assay at a concentration of 100 μM (data not shown). Therefore, the levels of NO production in the cell medium were measured in the presence of compounds **1**–**14** at serial diluted concentrations (0–100 μM). As shown in

Table S3, compounds **3**–**7, 9** and **10** exhibited potential NO inhibitory activity with IC_{50} values in the range from 6.71 ± 0.72 to 13.54 ± 0.78 μM, whereas the other compounds were inactive. Compounds **3** and **4** were the best NO inhibitors, with IC_{50} values of 6.71 ± 0.72, 8.97 ± 0.59 μM, respectively, compared to that of the positive control L-NMMA (32.51 ± 3.70).

3. Experimental

3.1. General

Optical rotation was measured on a Jasco P2000 polarimeter. IR spectrum was recorded on a Spectrum Two FT-IR spectrometer. CD spectra were recorded on a Chirascan spectrometer. HR-ESI-MS was acquired on an Agilent 6530 Accurate Mass Q-TOF LC/MS. NMR spectra were recorded on a Bruker 600 MHz and 500 MHz spectrometer. Preparative HPLC were run on an Agilent 1100 system including quaternary pump, autosampler, DAD detector, and preparative HPLC column YMC J'sphere ODS-H80 (4 μm, 20 × 250 mm). Isocratic mobile phase with the flow rate of 3.0 mL/min was used in pre-HPLC. The open column chromatography was performed using silica gel, reversed phase C18, diaion HP-20 or sephadex LH-20, as the stationary phase. Thin layer chromatography was carried out using pre-coated silica gel 60 F_{254} and RP-18 F_{254S} plates.

3.2. Plant material

The *Elsholtzia ciliata* (Thunb.) Hyl. was collected in Hoa Binh province, Vietnam during January, 2020, and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. A voucher specimen (NCCT-P118) was deposited at the Institute of Marine Biochemistry, VAST.

3.3. Extraction and isolation

The dried powder of *E. ciliata* (10.0 kg) was sonicated 3 times with MeOH (50 °C, 20 *L* × 3, each) to give a MeOH extract (EC, 500 g) after removal of the solvents. The EC extract was suspended with water (5.0 L) then partitioned with dichloromethane and then ethyl acetate to give dichloromethane (EC1, 200.0 g), ethylacetate (EC2, 50.0 g), and water layer (EC3). The EC2 extract was chromatographed on a silica gel column eluting with gradient solvent of dichloromethane/methanol (20/1, 10/1, 5/1, 0/1, v/v) to give four corresponding fractions, EC2A-EC2D. The EC2B fraction was chromatographed on an silica gel column eluting with dichloromethane/methanol/water (5/1/0.1, v/v/v) to obtain four fractions, EC2B1-EC2B4. The EC2B1 fraction was further chromatographed on an HPLC eluting with 30% acetonitrile in water to yield compounds **3** (5.2 mg, t_R 28.4 min) and **4** (4.5 mg, t_R 32.3 min). Compound **2** (16.1 mg, t_R 33.2 min) was isolated from EC2B3 by an HPLC eluting with 32% acetonitrile in water. The EC2B3 fraction was chromatographed on an HPLC using 30% acetonitrile in water as a eluent to get compound **13** (12.7 mg, t_R 25.0 min). The EC3 was chromatographed on a Diaion HP-20 column eluting with water to remove sugar components, then

increasing the concentrations of methanol in water (25%, 50%, 75% and 100%) to give four fractions, EC3A-EC3D, respectively. The EC3C (32.0 g) was separated on a silica gel column eluting with dichloromethane/methanol/water (5/1/0.1, v/v/v) to give nine fractions EC3C1-EC3C9. The EC3C3 fraction was chromatographed on the HPLC eluting with 16% acetonitrile in water to yield **9** (4.3 mg, t_R 49.9 min). The EC3C5 fraction was chromatographed on the HPLC using 22% acetonitrile in water to yield compounds **6** (14.0 mg, t_R 44.5 min) and **7** (20.5 mg, t_R 48.2 min). The EC3C6 fraction was chromatographed on the HPLC using 18% acetonitrile in water to get compounds **1** (7.0 mg, t_R 43.0 min), **8** (5.0 mg, t_R 52.5 min), and **12** (30.2 mg, t_R 56.1 min). The EC3C6 fraction was chromatographed on the HPLC using 15% acetonitrile in water as a eluent to yield compounds **11** (10.4 mg, t_R 33.8 min) and **9** (8.5 mg, t_R 43.2 min). Compound **5** (6.7 mg, t_R 33.8 min) and **14** (20.2 mg, t_R 30.4 min) was obtained from EC3C9 after purified on the HPLC using 18% acetonitrile in water as a eluent.

3.3.1. Elsholciliata A (1)

Colorless amorphous powder, mp. 231 – 233 °C; $[\alpha]_D^{25}$: + 37.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 222, 284 nm; IR (KBr): ν_{max} 3393 (broad), 2924, 1704, 1606, 1513, 1073, 1031 cm^{-1}; CD (MeOH, c 0.2 mg/mL) λ (θ: mdeg): 274 (+1.50), 314 (-0.53). HR-ESI-MS *m/z* 553.2030 $[M + {}^{35}Cl]^-$ (calcd. for $[C_{24}H_{38}O_{12}{}^{35}Cl]^-$, 553.2057), *m/z* 554.2072 $[M + {}^{35+37}Cl]^-$ (calcd. for $[C_{24}H_{38}O_{12}{}^{35+37}Cl]^-$, 554.2092), *m/z* 555.2024 $[M + {}^{37}Cl]^-$ (calcd. for $[C_{24}H_{38}O_{12}{}^{37}Cl]^-$, 555.2027), *m/z* 517.2252 [M - H]$^-$ (calcd. for $[C_{24}H_{37}O_{12}]^-$, 517.2291). ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data shown in Table S1

3.3.2. Elsholciliata B (2)

Colorless amorphous powder, mp. 198 – 200 °C; $[\alpha]_D^{25}$: – 13.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 290, 333 nm; IR (KBr): ν_{max} 3355 (broad), 2919, 1703, 1603, 1281, 1160, 1075 cm^{-1}; CD (MeOH, c 0.2 mg/mL) λ (θ: mdeg): 215 (-2.40), 282 (-0.42), 299 (+0.49). HR-ESI-MS *m/z* 672.1484 $[M + {}^{35}Cl]^-$ (calcd. for $[C_{32}H_{31}NO_{13}{}^{35}Cl]^-$, 672.1484), *m/z* 673.1547 $[M + {}^{35+37}Cl]^-$ (calcd. for $[C_{32}H_{31}NO_{13}{}^{35+37}Cl]^-$, 673.1517), *m/z* 674.1489 $[M + {}^{37}Cl]^-$ (calcd. for $[C_{32}H_{31}NO_{13}{}^{37}Cl]^-$, 674.1454), *m/z* 660.1682 $[M + Na]^+$ (calcd. for $[C_{32}H_{31}NO_{13}Na]^+$, 660.1693). ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data shown in Table S2.

3.4. Alkaline hydrolysis of 2

Compound ***2*** (3.0 mg) was dissolved in 3.0 mL solution of KOH (1.0 M) in methanol and heated at 60 °C for 2 h. Reaction was cooled to room temperature and carefully neutralised with solution of HCl 1.0 M. The solvent was then driven out under nitrogen flow. The residue was re-dissolved in 3.0 mL of water and extracted with ethyl acetate. The ethyl acetate extract was chromatographed on an HPLC (J'sphere H-80 column, 250 mm length x 20 mm ID, eluting with 20% acetonitrile in water, a flow rate of 3.0 mL/min) to give compounds **2c** (t_R 25.2, 0.7 mg), **2b** (t_R 29.4, 0.8 mg), and **2a** (t_R 36.7, 1.1 mg) (The physicochemical data of compounds **2a**, **2b**, and **2c** were shown in the Supporting Materials).

3.5. Td-DFT calculation of ECD spectra

TD-DFT calculation of ECD spectra were performed as previously described (Huyen et al. 2017). Stereoisomers (**2a**-*S*) and (**2b**-*S*) were generated and submitted for MMFF conformational search using Spartan 18. Conformations were then geometrically pre-optimised with semi-empirical PM3 set. Initial stable conformers with Boltzmann distribution over 1% were optimised by DFT with B3LYP/6-31G(d,p) basic set and polarisable continuum model (PCM) of methanol using Gaussian 16. Optimized conformers were continuously subjected to TDDFT calculation at the same basic set level and solvent effects. The ECD spectra at 30 excited states and half-band value at 0.3 eV for each conformer were summed based on their weighting Boltzmann distribution to yield theoretical ECD spectra of each stereoisomer. The calculated ECD spectra of (**2a**-*S*) and (**2b**-*S*) were UV-shifted by 5 and 7 nm, respectively. Calculated ECD spectra for **2a**-*R* and **2b**-*R* isomers were obtained by mirror imagine that of (**2a**-*S*) and (**2b**-*S*) spectra.

3.6. Acid hydrolysis and confirmation of monosaccharide

Compounds **1** and **2** (each 1.0 mg) were reacted with 2N aqueous HCl (1 ml) in sealed flask at 90 °C for 2 h. For each compound, the acidic aqueous mixtures was dried, added $CHCl_3$ (1 mL), and then this solution was extracted with H_2O (1 mL). The aqueous fraction was dried to get the sugar moieties. These sugars and the standard sugars, D-glucose, L-glucose and D-apiose (Sigma Aldrich) were dissolved separately in pyridine (1 mL), heated with L-cysteine methyl ester (2 mg) at 60 °C for 1 h, and then 2.5 μL *O*-tolyl isothiocyanate was added to the reaction mixture and further reacted at 60 °C for 1 h. The reaction mixture was analyzed on a HPLC using 250 × 4.6 mm i.d. Ultimate^{TM} XB-C18 column (Welch Material, Inc.) at 35 °C with isocratic elution of 25% CH_3CN in 0.5% formic acid for 40 min at a flow rate 0.8 ml/min, detected by UV detector (at 250 nm). Under these conditions, the standard sugars gave peaks at t_R (min) 22.7 (D-glucose), 21.8 (L-glucose), and 36.9 (D-apiose). Peaks at t_R (min) 22.7 (D-glucose) and 36.9 (D-apiose) for **1** and 22.7 (D-glucose) **2** were observed.

4. Conclusions

From the aerial parts of *Elsholtzia ciliata*, two new compounds named as elsholciliatas A and B together with twelve known ones were isolated. Their chemical structure were determined based on the extensive of UV, IR, 1D and 2D-NMR, HRESIMS experiments. The absolute configurations of the compounds **1** and **2** were successfully indicated by combination of both theoretical and calculated CD spectra as well as by chemical transformations. Compounds **3**-**7**, **9** and **10** potential inhibited NO production in LPS-activated RAW264.7 cells with IC_{50} values of 6.71 ± 0.72, 8.97 ± 0.59, 12.38 ± 0.42, 14.27 ± 1.12, 16.13 ± 0.98, 13.54 ± 0.78, 16.27 ± 0.91 μM, respectively, compared to that of the positive control of N^G-monomethyl-L-arginine acetate (L-NMMA), IC_{50} = 32.51 ± 3.70 μM.

Disclosure statement

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ORCID

Phan Van Kiem http://orcid.org/0000-0003-0756-6990

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