Glycoside constituents from *Miliusa sinensis* leaves and their anti-inflammatory and acetylcholine protective effects

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**ABSTRACT**

Repeated column chromatography resulted in the isolation of two new glycosides, miliusides A–B (1 and 7), along with six known metabolites (2–6, and 8) from the leaves of *Miliusa sinensis* Finet and Gagnep. The structures of the purified phytochemicals were elucidated by interpreting their spectroscopic data (NMR, HRMS), as well as comparison with the previous literature. The biological evaluation of acetylcholinesterase (AChE) inhibitory effects and anti-inflammatory activity by measuring nitric oxide (NO) production in lipopolysaccharide (LPS)-induced RAW264.7 mouse macrophage cells, were also conducted. Among them, compounds 5 and 7 exhibited significant AChE inhibitory activities (IC\textsubscript{50} = 53.36 ± 4.20 and 88.50 ± 8.79 μM, respectively), compared with the positive control (Galanthamine, IC\textsubscript{50} = 1.65 ± 0.15 μM). Only the MeOH extract showed suppression effects on NO production in LPS-induced RAW264.7 cells (IC\textsubscript{50} = 38.18 ± 3.25 μg/mL) comparable to that of the positive control, L-NMMA (IC\textsubscript{50} = 2.21 ± 0.56 μg/mL).
1. Introduction

*Miliusa sinensis* Finet and Gagnep. (syn.: *Euodia lyi* H. Lév.) is an evergreen tree up to 6 m tall, leaf blade elliptic, oblong-elliptic, or rarely oblong, most parts pubescent and densely so when young (Theplantlist.org 2021). This plant occurs in many Asia countries such as Vietnam, Thailand, and China (Chaowasku and Kessler 2014). In Vietnam, *M. sinensis* is found in the northern region of the country (Ho 1999), and its roots have been used in Chinese folk medicine for the treatment of bone fractures (Son 2019). Previous studies on the chemical and biological properties of this plant have resulted in the separation of a series of novel molecules with a rare C-18 skeleton containing a characteristic spiro-ring system, which was designated as miliusanes (Zhang et al. 2006; Xu et al. 2019). Interestingly, in the family Annonaceae, the serial novel miliusanes were reported only in the *Miliusa* genus, making it a significant chemotaxonomic marker of that specific genus. Also, the flavonoid constituents have been reported in only some cases (Thuy et al. 2011; Son 2019). Even though the current information relating to *M. sinensis* limited to the above study, other *Miliusa* species have been investigated extensively (Son 2019). Most previous studies were mainly focused on the constituents for the less polar fractions, and only a few megastigmane glycosides were obtained occasionally from the highly polar fractions of different *Miliusa* species (Thuy and Anh 2010; Son 2019). However, to date, there are no reports on the glycoside constituents from *M. sinensis*.

To elucidate the structural diversity and find novel and effective lead compounds, a detailed phytochemical investigation of *M. sinensis* leaves was conducted. Chromatographic separation of the water layer of a 95% methanolic extract led to the isolation of eight compounds (1–8, Figure 1). The isolation, structural elucidation, and biological evaluation of these compounds are reported herein.

![Figure 1. Structures of compounds 1–8 isolated from *M. sinensis* leaves.](image-url)
2. Results and discussion

Miliuside A (1) was obtained as a white, amorphous powder, with a negative optical rotation $[\alpha]_{D}^{24} = -140.2$ (c 0.15, MeOH). Its molecular formula was found to be C$_{18}$H$_{24}$O$_{11}$ (eight indices of hydrogen deficiency) as inferred from the HRESIMS (Figure S1) ion peak at m/z 451.1005 [M + Cl]$^-$ (calcd for C$_{18}$H$_{24}$ClO$_{11}$, 451.1007) and the $^{13}$C NMR data. The $^1$H NMR spectrum of 1 (Figure S3–S5) displayed signals assignable to four aromatic protons attributed to a 1,4-disubstituted aromatic ring [$\delta$H 7.25 (2H, d, J = 7.0 Hz, H-2/H-6) and 7.89 (2H, d, J = 7.0 Hz, H-3/H-5)], which were observed as an AABB system suggesting the presence of a p-disubstituted benzene ring. The presence of an aldehyde group was confirmed by the proton signal at $\delta$H 9.86 (1H, s, H-7). Moreover, the presence of two anomeric proton signals of H-1$' [\delta$H 5.01 (1H, d, J = 7.5 Hz)] and H-1$''$ [\deltaH 4.96 (1H, d, J = 2.5 Hz)] was attributed to a $\beta$-glucosyl and $\beta$-apiofuranosyl moiety. The $^{13}$C NMR spectrum of 1 in combination with HSQC spectroscopic data (Figure S6–S8) revealed the presence of 18 carbon signals, seven of which were assigned to a phenolic aglycone moiety, including one aldehyde [\deltaC 192.9 (C-7)] and six aromatic carbon atoms. This was confirmed the HMBC correlations (Figure S21) between $\delta$H 7.25 (H-2/H-6) with $\delta$C 164.0 (C-1), 117.9 (C-2/C-6), and 132.6 (C-4); between $\delta$H 7.89 (H-3/H-5) with $\delta$C 164.0 (C-1), 132.6 (C-4), and 192.9 (C-7); and $\delta$H 9.86 (H-7) with $\delta$C 132.6 (C-4) (Figure S21). The remaining 11 carbon signals of 1 suggested the presence of sugar moiety, which were confirmed as one hexose ($\beta$-D-glucose) and one pentose ($\beta$-D-apiose) unit (Table S1).

Careful inspection of the NMR spectra of compound 1 (Table S1) revealed that the $^1$H and $^{13}$C NMR data closely resembled those of 4-hydroxyacetophenone 4-O-[6$'$.O-$\beta$-D-apiofuranosyl]-$\beta$-D-glucopyranoside (Harput et al. 2004), except for the replacement of an acetoxy group with an aldehyde group in the aglycone moiety. The position of the aldehyde group at C-4 was verified by the HMBC correlation between $\delta$H 9.86 (H-7) and $\delta$C 132.6 (C-4) (Figure S21), as well as the upfield shift of C-7 (\deltaC 192.9) in 1 compared with those of 4-hydroxyacetophenone 4-O-[6$'$.O-$\beta$-D-apiofuranosyl]-$\beta$-D-glucopyranoside (\deltaC 199.8 (C-7), 26.6 (OAc)). In the $^{13}$C NMR spectroscopic data of 1 (Table S1), 11 carbons were clearly identified that corresponded to the disaccharide core formed by $\beta$-glucopyranosyl and $\beta$-apiofuranosyl units with an api(1$:$6)glc-linkage. The downfield shift of an oxygenated methylene carbon C-6$'$ (\deltaC 68.8) suggested glycosylation at this carbon, which was confirmed by the observed HMBC correlations ($^3$JCH) between $\delta$H 4.96 (d, J = 2.5 Hz, H-1$''$) with $\delta$C 68.8 (C-6$'$) and between $\delta$H 4.04 (dd, J = 11.0, 2.5 Hz, H-6$'$a)/3.63 (dd, J = 11.0, 5.5 Hz, H-6$'$b) with $\delta$C 111.0 (C-1$'$). Attachment of the sugar moiety at C-1 was also determined by an HMBC correlation of $\delta$H 4.01 (d, J = 7.5 Hz, H-1$'$) with $\delta$C 164.0 (C-1). Furthermore, the presence of $\alpha$-glucose and $\alpha$-apiose as sugar components in 1 was further confirmed by acid hydrolysis, converted to thio carbamoylthiazolidine derivative, and followed by HPLC analysis (Tanaka et al. 2007) in comparison with authentic d/l-glucose and apinose (Thao et al. 2015). From the above evidence, the structure of 1 was determined as 4-formylphenyl 1-O-[6$'$.O-$\beta$-D-apiofuranosyl]-$\beta$-D-glucopyranoside.

Miliuside B (7) was obtained as colorless needles. Its molecular formula was deduced to be C$_{21}$H$_{36}$O$_{8}$ by HRESIMS (Figure S10) ion peaks at m/z 439.2312 [M + Na]$^+$ (calcd for C$_{21}$H$_{36}$NaO$_{8}$, 439.2307) and $^{13}$C NMR data, requiring the presence
of four degrees of unsaturation. The $^1$H NMR data (Figure S12–S14 and Table S2) of 7 displayed the signals of four tertiary methyls \([\delta_H \, 1.11 \, (3H, \, s, \, H-12), \, 1.21 \, (3H, \, s, \, H-13), \, 1.24 \, (3H, \, s, \, H-14), \, \text{and} \, 1.33 \, (3H, \, s, \, H-15)]\), five methines \((\text{including an oxymethine} \, [\delta_H \, 3.38 \, (1H, \, m, \, H-8)])\), and two characteristic signals of a cyclopropane ring \([\delta_H \, 0.60 \, (1H, \, br \, d, \, J = 10.0 \, Hz, \, H-6) \text{and} \, 0.66 \, (1H, \, br \, d, \, J = 10.0 \, Hz, \, H-7)])\), as well as three methylenes \([\delta_H \, 1.76 \, (1H, \, m, \, H-2a)/1.64 \, (1H, \, m, \, H-2b), \, 1.95 \, (1H, \, m, \, H-3a)/1.48 \, (1H, \, m, \, H-3b), \, \text{and} \, 1.87 \, (2H, \, overlapped \, signals, \, H-9)])\). Additionally, the anomeric proton signal of H-10 \([\delta_H \, 4.46 \, (1H, \, d, \, J = 7.5 \, Hz)]\) suggested a sugar moiety binding to aglycone via $\beta$-glycosidic linkage \((a \, \text{trans\, diaxial configuration of H-1' \, and} \, H-2') \) from the large coupling constant \((3J_{1',2'} = 7.5 \, Hz)\). Analysis of the 1D NMR data (Figure S12–S16) of 7, as well as the HSQC spectrum, exhibited 21 carbon resonances, 15 of which were attributed to aglycone moiety, including four methyls, three methylenes, five methines \(\text{[one oxygenated at} \, \delta_C \, 67.3 \, (C-8)]\), and three none-protonated carbons \(\text{[including two oxygenated carbons at} \, \delta_C \, 89.0 \, (C-4) \, \text{and} \, 73.8 \, (C-10)]\) \((\text{Table S2})\). The remaining six-carbon signals could be assigned to the monosaccharide moiety that was clearly indicated from the 1D NMR data. These spectral data, coupled with the four degrees of unsaturation, suggested that compound 7 is a tricyclic sesquiterpenoid glycoside.

A comprehensive analysis of its NMR spectroscopic data indicated that its structure is closely related to that of alloaromadendrane-4$\beta$,10$\alpha$-diol \((\text{Moreira et al. 2003})\), which suggests that 7 is an aromadendrane-type sesquiterpenoid with the presence of an additional hydroxy group and a sugar unit. This suggestion was proved by a detailed analysis of 1D and 2D NMR data, especially the $^1$H-$^1$H COSY and HMBC spectrum. The subunit spin systems were established by the $^1$H-$^1$H COSY relationships from C-1 to C-3, from C-5 to C-9, and from C-5 to C-1 (Figure S19). Their connectivity of a five- and seven-membered ring system was fused at C-1 and C-5 and a three-membered ring was formed by C-6, C-7, and C-11 was determined through HMBC correlations. Further analysis of the HMBC relationships from $\delta_H \, 1.95 \, (H-3a)/1.48 \, (H-3b) \, \text{to} \, \delta_C \, 57.6 \, (C-1), \, 89.0 \, (C-4); \, \text{from} \, \delta_H \, 1.33 \, (H_3-15) \, \text{to} \, \delta_C \, 40.6 \, (C-3), \, 89.0 \, (C-4), \, \text{and} \, 48.4 \, (C-5); \, \text{and from} \, \delta_H \, 1.44 \, (H-5) \, \text{to} \, \delta_C \, 89.0 \, (C-4), \, 30.1 \, (C-6) \, \text{indicated the connection of the subunits to form a cyclopentane ring. The connection of the subunits to form a cycloheptane ring with two hydroxy groups located at C-8 and C-10 was confirmed by HMBC relationships from} \, \delta_H \, 1.44 \, (H-5) \, \text{to} \, \delta_C \, 30.1 \, (C-6), \, 21.1 \, (C-11); \, \text{from} \, \delta_H \, 0.60 \, (H-6) \, \text{to} \, \delta_C \, 89.0 \, (C-4), \, 34.5 \, (C-7), \, 28.8 \, (C-12); \, \text{from} \, \delta_H \, 1.87 \, (H-9) \, \text{to} \, \delta_C \, 57.6 \, (C-1), \, 34.5 \, (C-7), \, 67.3 \, (C-8), \, 73.8 \, (C-10); \, \text{and from} \, \delta_H \, 1.24 \, (H_3-14) \, \text{to} \, \delta_C \, 57.6 \, (C-1), \, 55.8 \, (C-9), \, \text{and} \, 73.8 \, (C-10). \, \text{Moreover, two hydroxy groups were linked to} \, C-8 \, \text{and} \, C-10 \, \text{matching well with the chemical shift at} \, \delta_C \, 67.3 \, \text{for the oxymethine (C-8) \, and} \, \delta_C \, 73.8 \, \text{for the oxygenated quaternary carbon (C-10), respectively. The geminal methyls were attached to the quaternary carbon C-11 position based on the HMBC correlations between} \, \delta_H \, 1.11 \, (H_3-12) \, \text{with} \, \delta_C \, 30.1 \, (C-6), \, 34.5 \, (C-7), \, 21.1 \, (C-11), \, 16.9 \, (C-13) \, \text{and between} \, \delta_H \, 1.21 \, (H_3-13) \, \text{with} \, \delta_C \, 30.1 \, (C-6), \, 34.5 \, (C-7), \, 21.1 \, (C-11), \, \text{and} \, 28.8 \, (C-12). \, \text{Finally, the connection of the aromadendrane-type and a sugar moiety was located at C-4, which was also supported by the HMBC correlations between the anomeric proton} \, \delta_H \, 4.46 \, (H-1') \, \text{to} \, \delta_C \, 89.0 \, (C-4) \, \text{(Figure S18). The monosaccharide in the sugar residue was further confirmed to be D-glucose by hydrolysis, convert to thiazolidine derivatives, HPLC analysis, and comparison retention time with that of standard monosaccharide derivative prepared in the same procedure (Tanaka et al. 2007).}
The relative configurations for 7 were mainly fixed by analysis of the NOESY spectrum. The NOESY relationship between H-6 and H-7 suggested a cis-fusion between the cycloheptane and cyclopropane rings, and H-6 and H-7 were arbitrarily assigned as α-oriented. The NOESY correlations of H-6 with H3-12/H3-15; H-7 with H3-12; H3-15 with H-6/H-7 showed that all were cofacial and were arbitrarily assigned to be α-oriented. The hydroxy group at C-8 was determined to be α-oriented by the correlations between H-8 and H-5/H-9 b/H3-13/H3-14. Besides, additional NOESY interactions from H-1 to H-2/β/H-5/H-14 indicated that H-1 and H-14 were also β-oriented. However, due to the flexibility of the seven-membered ring, the relative configuration of 7 could not be fully established from its NOESY data. Based on the above analysis, the structure of 7 was established as alloaromadendrane-4β,8α,10α-triol 4-O-β-D-glucopyranoside and was named miliuside B.

Other compounds were determined to be 3,4-dimethoxyphenol 1-O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (2) (Warashina et al. 2004), 3,4,5-trimethoxyphenol 1-O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (3) (Kanchanapooma et al. 2002), 6’-O-β-D-apiofuranosylthalictoside (4) (Wu et al. 2004), osmanthuside H (5) (Sugiyama and Kikuchi 1993), icariside D1 (6) (Miyase et al., 1987), and pteleifoside C (8) (Zhang et al. 2012) by their good consistency with those reported in the literature (Figure 1).

The cholinesterases are an important class of enzymes that catalyze the hydrolysis of ester bond linkages in choline-containing molecules. Biomedically, acetylcholinesterases play several important roles because of their abilities to rapidly hydrolyze ACh (a key neurotransmitter) and thus terminate nerve signal transmission. Consequently, the therapeutic inhibition of AChE using drugs such as donepezil, rivastigmine, and galanthamine is an effective strategy for symptomatic relief from clinical pathologies associated with several diseases including Alzheimer’s disease, Parkinson’s disease, myasthenia gravis, and glaucoma (Colović et al. 2013). AChE inhibitors are important medications that have received in the US. FDA approval for the treatment of mild to moderate Alzheimer’s disease. The search for AChE inhibitors from traditional medicines is an important way to find compounds aiming at preventing and treating this disease. Thus, compounds 2–8 were evaluated for their AChE inhibitory activities using galanthamine (IC50 = 1.65 ± 0.15 μM) as a positive control. Galanthamine derived from natural products (widely occurring in the Amaryllidaceae plants) is the commonly prescribed cholinergic enhancer as AChE inhibitors (Pohanka et al. 2008). The results (Table S3) revealed that 5 and 7 showed weak AChE inhibitory activities (IC50 = 53.36 ± 4.20 and 88.50 ± 8.79 μM, respectively), while compounds 2, 3, and 8 exhibited very weak AChE inhibitory activities, with IC50 values ranging from 124.88 ± 13.02 to 193.70 ± 9.85 μM. The remaining compounds did not exhibit significant AChE inhibitory activities (inhibition rates less than 50% at the concentration of 100 μM).

Inflammation, as a reaction to injury, infection, or internal stress, induces symptoms of redness, swelling, heat, and often pain. Long-lasting inflammation raises the risk of chronic diseases. Nitric oxide (NO) is a pro-inflammatory mediator that induces inflammation due to overproduction in abnormal situations (Choi and Hwang 2019). Under normal and physiological situations, NO is vital in the regulation of pathophysiological processes including neuronal signaling, neurotoxicity, and vasodilation. In contrast, abnormal production of NO can lead to tissue damage correlated with acute or
chronic inflammation (Coussens and Werb 2002). Therefore, inhibitors of NO production and such natural products with low cytotoxicity appear to offer a promising approach for the treatment of chronic inflammation. Lipopolysaccharide (LPS) is used as an activator of macrophages and involves the production of pro-inflammatory cytokines (Jin et al. 2019).

Many of the glycoside constituents isolated from the *Miliusa* genus are known to inhibit the production of nitric oxide (NO), which is a multifunctional signaling molecule related to vascular and neurological functions. Thus, the eight isolated compounds and the MeOH extract were evaluated for their *in vitro* anti-inflammatory activity by measuring nitric oxide (NO) production in LPS-induced RAW264.7 cells by a Griess assay (Khan et al. 2020). L-NMMA (N^G^-Methyl-L-arginine acetate salt) was employed as the positive control (IC_{50} = 2.21 ± 0.56 μg/mL). Cell viability was evaluated by the MTT assay, indicating that none of the tested compounds showed any significant cytotoxicity at their effective concentration for the inhibition of NO production (data not shown). The MeOH extract at 20 and 100 μg/mL exhibited potential anti-inflammatory properties by clearly inhibiting more than 30% NO production compared with non-treated cells. The MeOH extract produced 50% NO inhibition activity (IC_{50} = 38.18 ± 3.25 μg/mL). However, little effect was observed at the lower concentrations. All compounds showed weak NO production inhibitory activities (IC_{50} > 80 μM). The moderate biological activity seen in the extract of *M. sinensis* may be due to the presence of minor secondary metabolites that were not isolated in this study. Moreover, the possibility of moderate activity due to the synergistic effects of the various components of the extract cannot be ruled out.

### 3. Experimental

#### 3.1. General experimental procedures

Optical rotations were determined on a JASCO P-2000 polarimeter (Tokyo, Japan). IR spectra were obtained on a Bruker TENSOR 37 FT-IR spectrometer (Bruker, MA, USA). The \(^1\)H NMR (500 MHz) and \(^{13}\)C NMR (125 MHz) spectra were recorded on an AVANCE III HD 500 spectrometer (MA, USA) with tetramethylsilane (TMS) used as an internal standard. The HRESIMS were acquired on an Agilent 6530 Accurate-Mass Q-TOF LC/MS system (Emeryville, CA, USA). Medium pressure liquid chromatography (MPLC) was carried out on a Biotage-Isolera One system (SE-751 03 Uppsala, Sweden). Column chromatography (CC) was conducted using 65-250 or 230-400 mesh silica gel (Sorbent Technologies, Atlanta, USA), porous polymer gel (Diaion HP-20, 20-60 mesh, Mitsubishi Chemical, Tokyo, Japan), Sephadex LH-20 (Supelco, PA, USA), octadecyl silica (ODS, 50 μm, Cosmosil 140 C\(_{18}\)-OPN, Nacalai Tesque), and RP-18 (30-50 μm, YMC\(^\circledast\)GEL, Fuji Silysia Chemical). Analytical thin-layer chromatography (TLC) systems were performed on precoated silica gel 60 F\(_{254}\) (1.05554.0001, Merck) and RP-18 F\(_{254S}\) plates (1.15685.0001, Merck), and the isolated compounds were visualized by spraying with 10% H\(_2\)SO\(_4\) in water and then heating for 1.5–2 minutes. All procedures were carried out with solvents purchased from commercial sources that were used without further purification.
3.2. Plant material

The leaves of *Miliusa sinensis* Finet and Gagnep. were collected at Na Hang, Tuyen Quang province, Vietnam in March 2020, and taxonomically identified by Dr. Nguyen The Cuong (Institute of Ecology and Biological Resources, VAST). A voucher specimen (NCCB-01-MS) was deposited at the Herbarium of the Institute of Marine Biochemistry and Thuyloi University.

3.3. Extraction and isolation

The leaves of *M. sinensis* (2.5 kg) were dried, ground, and extracted with 95% aqueous MeOH (15 L) three-time (each × 1 day) at room temperature under reflux and filtered. The filtrate was combined and concentrated under reduced pressure using a rotavapor to obtain a MeOH extract (150 g), which was suspended in distilled water (1.5 L) and then successively solvent partitioned with *n*-hexane, dichloromethane (3 times each) to yield *n*-hexane (H, 23 g), dichloromethane (D, 30 g), and a water layer (W). By comparison with a TLC analysis of the three fractions that resulted from the solvent partitioning indicated the presence of metabolites in the water layer.

The water layer was applied to a Diaion HP-20 column and eluted with stepwise additions of MeOH in water (0%, 25%, 50%, 75%, 100%) to obtain four major subfractions (W.1 to W.4). In view of the identical TLC profiles, fraction W-2 (19 g) was successively separated on silica gel MPLC (column: Biotage SNAP Cartridge, KP-SIL, 100 g) using a mobile phase of CH$_2$Cl$_2$-MeOH (0–5 min 25% MeOH, 6–50 min 25–50% MeOH, 56–80 min 90% MeOH, 81–95 min 100% MeOH, 15 mL/min, 95 min) to obtain six subfractions (W-2.1 to W-2.6). Further purification of subfraction W-2.2 (0.7 g) was subjected to Sephadex LH-20 open CC with a solvent system MeOH-H$_2$O (1:1) and a silica gel CC, eluting with CH$_2$Cl$_2$-MeOH (20:1, v/v), resulting in the purification of 3 (5.2 mg) and 4 (6.8 mg). Fraction W-2.4 (5.8 g) was further applied to a silica gel column (200–300 mesh) using gradient mixtures of CH$_2$Cl$_2$-MeOH-H$_2$O (6:1:0.1, v/v) to yield four subfractions (W-2.4a to W-2.4d). One of these fractions, subfraction W-2.4a (0.8 g) was successively applied to an open RP-18 column and further subjected to a Sephadex LH-20 chromatography eluting with MeOH-H$_2$O (1:4) as mobile phase to yield 1 (1.8 mg) and 2 (6.5 mg). Additionally, subfraction W-2.4b (1.05 g) was rechromatographed on silica gel by MPLC using a MeOH-EtOAc gradient system (20–100%) to give three subfractions (W-2.4b1 to W-2.4b3). Compounds 7 (2.0 mg) and 8 (5.5 mg) were further obtained from subfraction W-2.4b1 by an open RP-18 column eluted with MeOH-H$_2$O (1:2.5, v/v). Finally, subfraction W-2.4b3 (0.17 g) was further purified by a Sephadex LH-20 column with MeOH solvent and a silica gel CC with CH$_2$Cl$_2$-EtOAc (4:1) as the mobile phase, resulting in the isolation of compounds 5 (4.5 mg) and 6 (6.5 mg).

3.3.1. Physical and spectroscopic data of the new compounds

**Miliuside A (1):** White, amorphous powder; [α]$^D_{[24]}$ = −140.2 (c 0.15, MeOH); IR (KBr) $\nu_{\text{max}}$ 3378, 2931, 2889, 1727, 1682, 1601, 1511, 1429, 1384, 1240, 1164, 1070, 833, and 642 cm$^{-1}$; $^1$H NMR (CD$_3$OD, 500 MHz) $\delta_H$ ppm: 7.25 (2H, d, $J = 7.0$ Hz, H-2/H-6), 7.89 (2H, d, $J = 7.0$ Hz, H-3/H-5), 9.86 (1H, s, H-7), Glc: 5.01 (1H, d, $J = 7.5$ Hz, H-1),
3.48 (1H, dd, $J = 9.0$, 7.5 Hz, H-2'), 3.46 (1H, br d, $J = 9.0$ Hz, H-3'), 3.36 (1H, t, $J = 9.0$ Hz, H-4'), 3.66 (1H, m, H-5'), 4.04 (1H, dd, $J = 11.0$, 2.5 Hz, H-6'a), 3.63 (1H, dd, $J = 11.0$, 5.5 Hz, H-6'b), **Api:** 4.96 (1H, d, $J = 2.5$ Hz, H-1''), 3.91 (1H, d, $J = 2.5$ Hz, H-2''), 3.97 (1H, d, $J = 10.0$ Hz, H-4''a), 3.75 (1H, d, $J = 10.0$ Hz, H-4''b), and 3.60 (2H, br d, $J = 10.5$ Hz, H-5''); **13C NMR (CD$_3$OD, 125 MHz)** $\delta$C ppm: 164.0 (C-1), 117.9 (C-2/C-6), 132.9 (C-3/C-5), 132.6 (C-4), 192.9 (C-7), **Glc:** 101.6 (C-1'), 74.8 (C-2'), 77.91 (C-3'), 71.6 (C-4'), 77.3 (C-5'), 68.8 (C-6'), **Api:** 111.0 (C-1''), 78.0 (C-2''), 80.5 (C-3''), 74.9 (C-4''), and 65.5 (C-5''); HRESIMS m/z 451.1005 [M+Cl]$^-$ (calcd for C$_{18}$H$_{24}$ClO$_{11}$, 451.1007).

**Miliuside B (7):** Colorless needles; $[\alpha]_D^{24}$ −110.4 (c 0.15, MeOH); IR (KBr) $\nu_{\text{max}}$ 3418, 2924, 2855, 1624, 1459, 1381, 1076, 761 cm$^{-1}$; $^1$H NMR (CD$_3$OD, 500 MHz) $\delta$H ppm: 1.78 (1H, m, H-1), 1.76 (1H, m, H-2a), 1.64 (1H, m, H-2b), 1.95 (1H, m, H-3a), 1.48 (1H, m, H-3b), 1.44 (1H, m, H-5), 0.60 (1H, br d, $J = 10.0$ Hz, H-6), 0.66 (1H, br d, $J = 10.0$ Hz, H-7), 3.38 (1H, m, H-8), 1.87 (2H, overlapped signals, H-9), 1.11 (3H, s, H-12), 1.21 (3H, s, H-13), 1.24 (3H, s, H-14), 1.33 (3H, s, H-15), **Glc:** 4.46 (1H, d, $J = 7.5$ Hz, H-1'), 3.12 (1H, br d, $J = 9.0$ Hz, H-2'), 3.33 (1H, dd, $J = 9.0$, 9.0 Hz, H-3'), 3.29 (1H, t, $J = 9.0$ Hz, H-4'; 3.23 (1H, ddd, $J = 11.5$, 9.5, 2.5 Hz, H-5'), 3.83 (1H, dd, $J = 11.5$, 2.5 Hz, H-6'a), and 3.66 (1H, dd, $J = 11.5$, 5.5 Hz, H-6'b); $^{13}$C NMR (CD$_3$OD, 125 MHz) $\delta$C ppm: 57.6 (C-1), 25.5 (C-2), 40.6 (C-3), 89.0 (C-4), 48.4 (C-5), 30.1 (C-6), 34.5 (C-7), 67.3 (C-8), 55.8 (C-9), 73.8 (C-10), 21.1 (C-11), 28.8 (C-12), 16.9 (C-13), 21.0 (C-14), 22.1 (C-15), **Glc:** 99.2 (C-1'), 75.1 (C-2'), 78.6 (C-3'), 71.8 (C-4'), 77.8 (C-5'), and 62.9 (C-6'); HRESIMS m/z 439.2312 [M+Na]$^+$ (calcd for C$_{21}$H$_{36}$NaO$_8$)$^+$, 439.2307), and 451.2096 [M+Cl]$^-$ (calcd for C$_{21}$H$_{36}$ClO$_8$)$^-$, 451.2099).

### 3.4. Sugar identification

Determination of the absolute configuration of sugar components of 1 and 7 was conducted according to published protocols with modifications, as detailed in a previous paper (Thao et al. 2015) and described in supporting data.

### 3.5. Preparation of compounds for the biological evaluation

From the methanolic extract of *M. sinensis*, eight compounds (1–8) were isolated and structurally elucidated. Stock solutions of tested compounds in DMSO were prepared kept at −20°C and diluted to the final concentration in fresh media before each experiment. To not affect the cell growth, the final DMSO concentration did not exceed 0.5% in all experiments.

### 3.6. Acetylcholinesterase inhibitory activity testing

The AChE inhibitory activities of 2–8 were evaluated using Pohanka’s method with a slight modification (Pohanka et al. 2008) and described in supporting data.
3.7. **Anti-inflammatory assay**

The anti-inflammatory activity of the isolated compounds was evaluated for their inhibitory activity against LPS-induced NO production in the RAW264.7 macrophage cell line as previously published (Khan et al. 2020) and described in supporting data.

4. **Conclusions**

In summary, the phytoconstituents of *M. sinensis* leaves have been demonstrated in the present study, eight compounds including two new ones, miliusides A-B (1 and 7) were isolated. Their structures were elucidated by analysis of HRESIMS, NMR spectral data, and comparison with those reported in the literature. Phenolic glycosides were found to be the major constituents. In addition, compounds 5 and 7 showed inhibitory effects on AChE, with the IC\textsubscript{50} values of 53.36 ± 4.20 and 88.50 ± 8.79 μM, respectively. To the best of our knowledge, this represents the first report of acetylcholinesterase inhibitory activity from *Miluisa* species.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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