RESEARCH ARTICLE

A bioactive cycloartane triterpene from *Garcinia hombroniana*

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**ABSTRACT**

The dichloromethane bark extract of *Garcinia hombroniana* yielded one new cycloartane triterpene; (22Z,24E)-3β-hydroxycycloart-14,22,24-trien-26-oic acid (1) together with five known compounds: garcihombronane G (2), garcihombronane J (3), 3β-acetoxy-9α-hydroxy-17,14-friedolanostan-14,24-dien-26-oic acid (4), (22Z, 24E)-3β, 9α-dihydroxy-17,14-friedolanostan-14,22,24-trien-26-oic acid (5) and 3β, 23α-dihydroxy-17,14-friedolanostan-8,14,24-trien-26-oic acid (6). Their structures were established by the spectral techniques of NMR and ESI-MS. These compounds together with some previously isolated compounds; garcihombronane B (7), garcihombronane D (8) 2,3’4,5’-tetrahydroxy-6-methoxybenzophenone (9), volkensiflavone (10), 4’-O-methyl-volkensiflavone (11), volkensiflavone-7-O-glucopyranoside (12), volkensiflavone-7-O-rhamnopyranoside (13), Morelloflavone (14), 3’-O-methyl-morelloflavone (15) and morelloflavone-7-O-glucopyranoside (16) were evaluated for cholinesterase enzymes inhibitory activities using acetylcholinesterase and butyrylcholinesterase. In these activities, compounds 1–9 showed good dual inhibition on both the enzymes while compounds 10–16 did not reasonably contribute to both the cholinesterases inhibitory effects.

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1. Introduction

*Garcinia hombroniana*, belonging to family Clusiaceae, is known as manggis hutan (seashore mangosteen) in Malaysia or Waa in Thailand (Nazre 2010). In Malaysia, it is found in the coastal regions, from the lowland forests near the sea to the lower mountain forests and the highlands (Xiwen et al. 2007). The fruits, leaves, flowers, stem and bark of *Garcinia* species have been used traditionally to treat abdominal pain, dysentery, diarrhoea, suppuration, infections, leucorrhoea, chronic ulcer and gonorrhoea (Braide 1993; Moongkarndi et al. 2004; Balemba et al. 2010). Phytochemical investigation of *Garcinia* species revealed the isolation of xanthones, benzophenones, flavonoids, biflavonoids and triterpenes (Hiroyuki et al. 1996; Rukachaisirikul et al. 2000, 2003, 2005; Klailkay et al. 2013; Jamila, Khairuddeen, Khan, et al. 2014, Jamila, Khairuddeen, Yaacob, et al. 2014; Jamila, Khairuddeen, et al. 2015, Jamila, Yeong, et al. 2015) showing significant antimicrobial, antioxidant, cytotoxic, anti-inflammatory, anti-tumor, antiulcer and antiplasmodial activities (Gustafson et al. 1992; Sang et al. 2002; Ito et al. 2003; Matsumoto et al. 2003; Jayaprakasha et al. 2006; Mahabusarakam et al. 2006; Franklin et al. 2009). Natural triterpenes have shown potent antimicrobial, anti-inflammatory, anticarcinogenic, hepatoprotective, hypolipidaemic and anti-HIV activities (Rios et al. 2000; Takao & Midori 2000; Dzubak et al. 2006). There is limited study on the cholinesterase inhibitory activities of natural triterpenes. For example, the pharmacological studies of Ozturk et al. (2011) on *Micromeria ciliicica* showed the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of ursolic acid. Similarly, Rahman et al. (2001) reported on seven AChE- and BChE-inhibiting triterpenoidal alkaloids from *Buxus papillosa*. However, phenolic compounds, such as curcumin and resveratrol, and their analogues are extensively studied for their promising cholinesterase inhibitory activities (Hamagushi et al. 2010; Csuk et al. 2013). Previous phytochemical and pharmacological investigation on *G. hombroniana* resulted in the isolation of xanthones, benzophenones, flavonoids and triterpenes with cytotoxic, anticholinesterase, low density lipoprotein antioxidation and antiplatelet aggregation activities (Rukachaisirikul et al. 2000, 2003, 2005; Saputri & Jantan 2012; Klaiklay et al. 2013; Jamila, Khairuddeen, et al. 2015; Jamila, Yeong, et al. 2015). Previous phytochemical investigation on the bark of *G. hombroniana* afforded cholinesterase inhibitory and cytotoxic triterpenoidal constituents (Jamila, Khairuddeen, Yaacob, et al. 2014; Jamila, Khairuddeen, et al. 2015, Jamila, Yeong, et al. 2015). In our continued research, an investigation was undertaken on the dichloromethane bark extract of *G. hombroniana* which led to the isolation of one new cycloartane triterpene; (22Z,24E)-3β-hydroxycycloart-14,22,24-trien-26-oic acid (1) shown in Figure 1. Besides, five known triterpenoids were also isolated and identified as garcihombranone G (2), garcihombranone J (3), 3β-acetoxy-9α-hydroxy-17,14-friedolanostan-14,24-dien-26-oic acid (4), (22Z, 24E)-3β, 9α-dihydroxy-17,14-friedolanostan-14,22,24-trien-26-oic acid (5), 3β, 23α-dihydroxy-17,14-friedolanostan-8,14,24-trien-26-oic acid (6). These compounds, together with some previously isolated compounds (Figure 1); garcihombranone B (7), garcihombranone D (8); 2,3′,4′,5′-tetrahydroxy-6-methoxybenzophenone (9) by Rukachaisirikul et al. (2000) and Jamila et al. (2014b), volkensiflavone (10), 4″-O-methyl-volkensiflavone (11), volkensiflavone-7-O-glucopyranoside (12), volkensiflavone-7-O-rhamnopyranoside (13), morelloflavone (14), 3″-O-methyl-morelloflavone (15) and morelloflavone-7-O-glucopyranoside (16) by Jamila et al. (2014a) were evaluated for cholinesterase enzymes inhibitory activities using AChE and BChE.
2. Results and discussion

2.1. Isolation, characterisation and structure elucidation

The separation of dichloromethane bark extract of *G. hombroniana* over silica gel column chromatography (CC), yielded one new compound which was identified as (22,24E)-3β-hydroxycycloart-14,22,24-trien-26-oic acid (1) and few known constituents; garcihombrinane G (2), garcihombrinane J (3) 3β-acetoxy-9α-hydroxy-17,14-friedolanostan-14,24-dien-26-oic acid (4), (22Z, 24E)-3β, 9α-dihydroxy-17,14-friedolanostan-14,22,24-trien-26-oic acid (5), 3β, 23α-dihydroxy-17,14-friedolanostan-8,14,24-trien-26-oic acid (6) (Rukachaisirikul et al. 2005; Klaiklay et al. 2013). Compound 1 was obtained as a white crystalline solid with a

![Chemical structures of compounds 1–16.](image)
melting point of 183–185 °C. The TLC analysis of 1 gave a pink colour spot with Liebermann–Burchard reagent and 95% methanolic H₂SO₄ and purple colour with vanillin–sulphuric acid stain which are the characteristics of triterpenes. The molecular formula of 1 was determined to be C₃₀H₄₄O₃ with 9° of unsaturation deduced from electron impact (EI) and electrospray ionisation (ESI) mass spectrometry (Figures S1–S3 in supplementary material). EI, positive low-resolution and negative high-resolution ESI, respectively, exhibited molecular ion peaks; [M]⁺ at m/z 452.3, [M + H]⁺ at m/z 452.94 and [M–H]⁻ at m/z 451.2878 (calcd. 451.3290). The
IR spectrum of 1 demonstrated absorption bands at 3463 and 1685 cm\(^{-1}\) corresponding to O–H and C=O groups stretching, respectively. Apart from the olefinic proton (H-15) in the tetracyclic system which appeared at \(\delta H\) 4.89 as a broad singlet, \(^1\)H NMR spectrum (Table S1 and Figure S4 in supplementary material) also showed signals of three other olefinic protons at \(\delta H\) 7.40 (d, \(J = 12.0\) Hz, H-24), 6.24 (t, \(J = 11.5\) Hz, H-23) and 5.95 (t, \(J = 11.0\) Hz, H-22). These data suggested a side chain of the type CH(Me)CH=CH–CH=C(Me)COOH in 1. This was further confirmed by \(^1\)H-\(^1\)H COSY correlations (Figure S5 in supplementary material) of an olefinic proton at \(\delta H\) 7.40 (H-24) with an olefinic proton at \(\delta H\) 6.24 (H-23) and H-23 with another olefinic proton at \(\delta H\) 5.95 (H-22), confirming the presence of conjugated double bonds at C-22, C-23, C-24 and C-25 in a side chain. Besides, \(^3\)J cross-peaks in HMBC spectrum (Table S1 and Figure S6 in supplementary material) of H-22 (\(\delta H\) 5.95) to C-24 (\(\delta C\) 131.5), H-23 (\(\delta H\) 6.24) to C-20 (\(\delta C\) 38.5) and C-25 (\(\delta C\) 127.8), and H-24 (\(\delta H\) 7.40) to C-22 (\(\delta C\) 142.6), C-26 (\(\delta C\) 169.4) and C-27 (\(\delta C\) 12.3) supported the proposed side chain. Direct connectivities of a doublet of the downfield proton at \(\delta H\) 7.40 in HMQC spectrum (Figure S7 in supplementary material) with a carbon at \(\delta C\) 131.5 (C-24), a triplet at \(\delta H\) 6.24 with carbon at \(\delta C\) 121.4 (C-23) and another triplet at \(\delta H\) 5.95 with carbon at \(\delta C\) 142.6 (C-22) further confirmed the presence of side chain in 1. Position of the carboxylic (COOH) carbon as C-26 was determined by HMBC cross-peaks of H-24 (\(\delta H\) 6.83) with C-27 (\(\delta C\) 12.8) further strengthened the presence of side chain and carboxylic group position. In addition, two broad doublets of one oxymethine proton at \(\delta H\) 3.10 (H-3) with a \(J\) value of 10.5 Hz and of hydroxyl group (3-OH) at \(\delta H\) 4.43, and six singlets of methylated protons at \(\delta H\) 1.84, 0.95, 0.89, 0.87, 0.75 and 0.55 were also found in \(^1\)H NMR spectrum. There were typical signals of pair of doublets at \(\delta H\) 0.68 (d, \(J = 3.0\) Hz) and \(\delta H\) 0.42 (d, \(J = 3.0\) Hz) in \(^1\)H NMR spectrum, representing methylene protons of the cyclopropane ring in cycloartane triterpenes (Magadula 2010; Nguyen et al. 2011; Mohamed 2014). The signals of the rest of protons were superimposed resulting in complex overlapped multiplicities and were interpreted on the basis of HMQC and HMBC experiments. The \(^{13}\)C NMR spectrum (Table S1 and Figure S8 in supplementary material) showed signals of 30 carbons consisting of eight quaternary, eight methine, eight methylene and six methyl carbons, which were substantiated by the analysis of DEPT 135 and 90 NMR spectra (Figures S9 and S10 in supplementary material). The relative configurations of 1 were determined by ROESY spectrum (Table S1 and Figure S11 in supplementary material) in which H-3 (\(\delta H\) 3.10) showed correlations with H$_{3-29}$ (\(\delta H\) 0.89) and H-5 (\(\delta H\) 2.44). This was further supported by the coupling constant (10.5 Hz) of H-3 which indicated its \(\alpha\)-orientation, and that OH group is \(\beta\)-oriented. H$_{3-29}$ showed correlations with H-3 and H-5, confirming their cis configurations. H-5 showed correlations with H$_{3-29}$ which revealed that all these protons are cis. In addition, H$_{3-18}$ (\(\delta H\) 0.87) did not show any correlation with H-5 and H$_{3-30}$ (\(\delta H\) 0.55), showing that H$_{3-18}$ is trans to both H-5 and H$_{3-30}$. A cross-peak of H$_{3-30}$ with H$_{3-21}$ (\(\delta H\) 0.95) appeared which suggested that H$_{3-30}$ and H$_{3-21}$ are located at the same side of molecule. Thus, based on 1D and 2D NMR spectral data, mass spectrometry and comparison with related literature (Magadula 2010; Nguyen et al. 2011; Mohamed 2014), compound 1 was identified as \((22Z,24E)-3\beta\)-hydroxyoocyloart-14,22,24-trien-26-oic acid, a new cycloartane triterpene. Known compounds 2–6 were identified by the comparison of their spectroscopic data with the literature values (Rukachaisirikul et al. 2000, 2003, 2005; Klaiklay et al. 2013).
2.2. In vitro anticholinesterase activities

Compounds isolated in the current studies (1–6) and that isolated previously (7–16) were evaluated for anticholinesterase activities. In this investigation, only compounds 1–9 displayed more than 50% inhibition. Compounds 10–16 which showed less than 50% inhibition were considered inactive. Compound 9 was the significant inhibitor of AChE (IC\textsubscript{50} 10.3 μM) compared to the reference drug, galanthamine (IC\textsubscript{50} 2.05 μM), followed by 7, 4 and 1 which, respectively, showed an inhibition against AChE with the IC\textsubscript{50} of 16.3, 16.7 and 17.9 μM. Compounds 3 and 8 (IC\textsubscript{50} = 27.9 and 25.0 μM) were almost two times less active than compounds 9 and 7. In case of inhibitory activity against BChE, compounds 4, 5 and 7 showed good potency with IC\textsubscript{50} values of 16.1, 19.7 and 21.5 μM comparable to galanthamine (IC\textsubscript{50} = 19.24 μM). Compounds 1–3, and 6 and 9 in BChE inhibition showed only moderate activity with IC\textsubscript{50} of 28.4, 25.0, 31.4, 34.6 and 31.5 μM. In comparison with physostigmine, these compounds (1–9) showed less inhibition against AChE and BChE both. It is also worth to mention that compounds 1, 3 and 6–9 showed more selectivity towards AChE with selectivity indices of 1.58, 1.12, 1.47, 1.31, 1.58 and 3.05, while compounds 2 and 4 were more selective for BChE with selectivity indices of 1.40 and 1.03, respectively. Table 1 summarises the detailed % inhibition, IC\textsubscript{50} and the selectivity indices of tested compounds and standard drugs. AChE-specific inhibitors might be useful at the earlier stages of AD whereby AChE enzyme has dominant role in splitting of acetylcholine. However, as the disease progresses, the role of BChE becomes more prominent and therefore a dual inhibitor is expected to be beneficial at both stages.

Table 1. Anticholinesterase activities (% inhibition, IC\textsubscript{50}, selectivity indices) of compounds 1–16.

| Compound | % Inhibition at 50 μg/mL | IC\textsubscript{50} (μM) | % Inhibition at 50 μg/mL | IC\textsubscript{50} (μM) | Selectivity |
|----------|--------------------------|---------------------------|--------------------------|---------------------------|-------------|
|          | AChE                     | BChE                      | AChE                     | BChE                      |             |
| 1        | 79.5\textsuperscript{m}  | 17.9\textsuperscript{a}  | 68.2\textsuperscript{i}  | 28.4\textsuperscript{i}  | 1.58\textsuperscript{f}  |
| 2        | 67.2\textsuperscript{a}  | 35.1\textsuperscript{i}  | 75.1\textsuperscript{m}  | 25.0\textsuperscript{m}  | 0.712\textsuperscript{a}  |
| 3        | 74.1\textsuperscript{h}  | 27.9\textsuperscript{j}  | 70.9\textsuperscript{a}  | 31.4\textsuperscript{h}  | 1.12\textsuperscript{d}  |
| 4        | 73.8\textsuperscript{a}  | 16.7\textsuperscript{j}  | 78.4\textsuperscript{a}  | 16.1\textsuperscript{b}  | 0.964\textsuperscript{a}  |
| 5        | 69.8\textsuperscript{a}  | 21.2\textsuperscript{m}  | 74.0\textsuperscript{a}  | 19.7\textsuperscript{d}  | 0.929\textsuperscript{a}  |
| 6        | 72.6\textsuperscript{a}  | 23.5\textsuperscript{h}  | 61.9\textsuperscript{a}  | 34.6\textsuperscript{a}  | 1.47\textsuperscript{a}  |
| 7        | 79.3\textsuperscript{m}  | 16.3\textsuperscript{i}  | 70.0\textsuperscript{a}  | 21.5\textsuperscript{j}  | 1.31\textsuperscript{a}  |
| 8        | 78.7\textsuperscript{a}  | 25.0\textsuperscript{i}  | 66.2\textsuperscript{a}  | 39.7\textsuperscript{a}  | 1.58\textsuperscript{a}  |
| 9        | 81.3\textsuperscript{a}  | 10.3\textsuperscript{i}  | 59.0\textsuperscript{a}  | 31.5\textsuperscript{i}  | 3.05\textsuperscript{a}  |
| 10       | 28.6\textsuperscript{a}  | ND                        | 2.09\textsuperscript{a}  | ND                        | –            |
| 11       | 12.8\textsuperscript{a}  | ND                        | 19.8\textsuperscript{a}  | ND                        | –            |
| 12       | 4.46\textsuperscript{a}  | ND                        | 7.38\textsuperscript{a}  | ND                        | –            |
| 13       | 2.35\textsuperscript{a}  | ND                        | 5.24\textsuperscript{a}  | ND                        | –            |
| 14       | 18.1\textsuperscript{a}  | ND                        | 22.6\textsuperscript{a}  | ND                        | –            |
| 15       | 2.96\textsuperscript{a}  | ND                        | 9.91\textsuperscript{a}  | ND                        | –            |
| 16       | 1.85\textsuperscript{a}  | ND                        | 2.37\textsuperscript{a}  | ND                        | –            |
| Galanthamine\textsuperscript{a} | 2.05\textsuperscript{a} | 19.24\textsuperscript{e} | 9.38\textsuperscript{a} | 0.106\textsuperscript{b} |
| Physostigmine\textsuperscript{a} | 0.037\textsuperscript{a} | 0.091\textsuperscript{a} | 2.40\textsuperscript{a} | 0.41\textsuperscript{d} |

Notes: Superscripts (a–n) are significantly different at \(p<0.05\).

\textsuperscript{a}Selectivity for AChE is defined as IC\textsubscript{50}(BChE)/IC\textsubscript{50}(AChE).

\textsuperscript{b}Selectivity for BChE is defined as IC\textsubscript{50}(AChE)/IC\textsubscript{50}(BChE).

\textsuperscript{c}Positive control; ND = not detected.
3. Experimental

3.1. Plant materials
The plant (G. hombroniana) was collected from Penang Botanical Garden, Malaysia, and has been deposited at the herbarium of this Garden with a voucher specimen (PBGK12).

3.2. General experimental procedure
Silica gel 60 (0.040–0.063 mm) was used as an adsorbent for CC. Merck TLC pre-coated aluminium plates, silica gel 60 F254, were used for partitioning and detection. Melting points were determined using a Stuart Scientific Melting Point SMP 1 (UK). UV spectra were measured using a Perkin–Elmer, Lambda 25 UV/Vis spectrometer. IR spectra were recorded in KBr and also by direct placement of finely powdered samples on the eye of the Perkin–Elmer (USA) 2000 FT-IR spectrophotometer. One-dimensional and two-dimensional NMR experiments were performed at room temperature using a Bruker Ascend 500 MHz (1H) and 125 MHz (13C) spectrometer (Bruker Biospin, Switzerland). A Tecan Infinite 200 Pro Microplate spectrometer was used for the evaluation of the cholinesterase inhibitory assays.

3.3. Isolation and purification
A 10.0 g dark green dichloromethane extract from the air-dried ground 3.5 kg bark of G. hombroniana was subjected to silica gel (230–400 mesh, 900 g) CC. Elution was carried out using solvent systems of n-C6H14/EtOAc and EtOAc/MeOH in a polarity gradient manner (1:0 → 0:10). Six major combined fractions (DFA1–DFA6) of the 35 (DF1–DF35) fractions were obtained and separated using silica gel CC. The sub-fraction DFA2 (1.1 g) of purple spots on TLC after treating with 5% methanolic H2SO4 was preceded to silica gel CC with solvent systems of n-hexane, n-hexane/EtOAc and EtOAc, afforded compound 1 with 2:3 (v/v) n-hexane/EtOAc which was further recrystallised from a mixture of methanol and chloroform (9.5:0.5) few times. Sub fractions DF3, DF4 and DF5 on further fractionation afforded compounds 2 and 4, 3, 5 and 6 with n-hexane/EtOAc (1:9 → 0:10).

(22Z,24E)-3β-hydroxycycloart-14,22,24-trien-26-oic acid (1): white solid; mp: 183–185 °C; +HRESI-MS: m/z 451.2878 (calcd. 451.3290) [M–H]+ (calculated for C30H44O3); IR (KBr) V cm−1: 3463, 1685; 1H-NMR (DMSO-d6, 500 MHz): δH 7.40 (t, J = 12.0, 1H, H-24), 6.24 (t, J = 11.5, 1H, H-23), 5.95 (t, J = 11.0 Hz, 1H, H-22), 4.89 (br. s, 1H, H-15), 3.10 (br. d, J = 10.5, 1H, H-3), 2.92 (m, 1H, H-20), 2.44 (m, 1H, H-5), 1.95 (m, 1H, H-6a), 1.84 (s, 3H, H-27), 1.73 (m, 2H, H-7a, H-16a), 1.67 (m, 3H, H-1, H-2a), 1.60 (m, 1H, H-11a), 1.53 (m, 2H, H-12), 1.50 (m, 2H, H-6b, H-16b), 1.35 (m, 1H, H-2b), 1.29 (m, 1H, H-8), 1.20 (m, 1H, H-11b), 1.17 (m, 1H, H-7b), 0.95 (s, 3H, H-21), 0.89 (s, 3H, H-29), 0.87 (s, 3H, H-30), 0.75 (s, 3H, H-28), 0.68 (d, J = 3.0, 1H, H-19a), 0.55 (s, 3H, H-18), 0.42 (d, J = 3.0, 1H, H-19b); 13C-NMR (DMSO-d6, 125 MHz): δC 169.4 (C-26), 142.6 (C-22), 141.8 (C-14), 131.5 (C-24), 127.8 (C-25), 121.4 (C-23), 114.1 (C-15), 76.3 (C-3), 49.4 (C-5), 44.6 (C-13), 40.4 (C-8), 39.4 (C-4), 38.5 (C-20), 37.1 (C-17), 31.7 (C-12), 30.7 (C-1), 29.7 (C-11), 26.6 (C-19), 26.1 (C-7), 25.3 (C-10, C-29), 22.4 (C-2), 21.2 (C-6), 19.3 (C-9), 17.3 (C-30), 16.1 (C-21), 13.2 (C-18), 12.8 (C-28), 12.3 (C-27). See also Table S1 for 1H, 13C, HMBC and ROESY-NMR data in Supplementary Material.

3.4. Evaluation of cholinesterase inhibitory activities

3.4.1. Chemicals, enzymes and reagents
AChE from electric eel, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCI), BChE from equine serum, S-butyrylthiocholine chloride, physostigmine and
galanthamine hydrobromide were purchased from Sigma (St Louis, MO, USA). Deionised water was prepared in-house using Maxima ultra-pure water system from ELGA (Bucks, UK).

3.4.2. Cholinesterase inhibitory assay
Cholinesterase inhibitory activities of the compounds 1–14 were determined by slightly modified Ellman’s assay (Ellman et al. 1961). In this assay, 140 μL of 0.1 M sodium phosphate buffer (pH 8.0) was added to 96 wells microplate followed by 20 μL of test samples and 20 μL of 0.09 units/mL AChE enzyme. After 15 min of pre-incubation at room temperature, 10 μL of 10 mM DTNB was added into each well following the addition of 10 μL of 14 mM ATCl. Absorbance of the coloured end product was measured using Tecan Infinite 200 Pro Microplate spectrometer at 412 nm at 30 min after initiation of the enzymatic reaction. Absorbance of the test samples was corrected by subtracting the absorbance of their respective blank. BChE inhibitory assay was carried out with the same procedure using the BChE enzyme and S-butyrylthiocholine chloride as substrate. Galanthamine and Physostigmine were taken as reference standards. The test samples and the standards were prepared in DMSO at the initial concentration of 1 mg/mL. The concentration of DMSO in final reaction mixture was 1%. Initial cholinesterase inhibitory activity of the compounds was evaluated at 50 μg/mL. Compounds with 50% or more inhibition of concentrations (50.0, 25.0, 12.5, 6.25 and 3.125 μM) were further evaluated for the determination of 50% inhibitory concentration (IC50).

3.5. Statistical analyses
All data were analysed and expressed as means ± standard deviation of three replicates. The differences between the assayed values were analysed using one-way analysis of variance, followed by Tukey’s HSD test at 95% and 99% confidence interval with SPSS software, version 19.0 (SPSS Inc., Chicago, USA). IC50 were calculated using GraphPad Prism 6.02 (GraphPad Software Inc., La Jolla, USA).

4. Conclusions
In this study, one new cycloartane triterpene and five known triterpenoids were isolated from dichloromethane extract of G. hombroniana. These compounds and some previously isolated triterpenes, benzophenone and biflavonoids from G. hombroniana were evaluated for cholinesterase enzymes inhibitory activities. In this in vitro enzymatic study, only compounds 1–9 showed moderate cholinesterase inhibitory effects with more than 50% inhibition against both AChE and BChE. Compound 9 was the significant inhibitor of AChE followed by 7, 4 and 1. In BChE inhibition, compounds 4 and 5 showed good potency comparable to galanthamine. In view of the obtained results, it can be concluded that further future phytochemical and pharmacological studies using advanced experimental techniques on the bark of G. hombroniana is needed.

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Disclosure statement
No potential conflict of interest was reported by the authors.
### Supplementary data and research materials

$^{1}$H, $^{13}$C, HMBC and ROESY-NMR tabulated data, EI/ESI-MS, $^{1}$H NMR, $^{13}$C NMR, DEPT 135 and DEPT 90 NMR, COSY, HMQC, HMBC and ROESY spectra of compound 1, $^{13}$C NMR spectra of some known compounds, and ORTEP views of compounds 7 and 8 are available as supplementary material at [http://dx.doi.org/10.1080/14786419.2015.1060594](http://dx.doi.org/10.1080/14786419.2015.1060594).

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