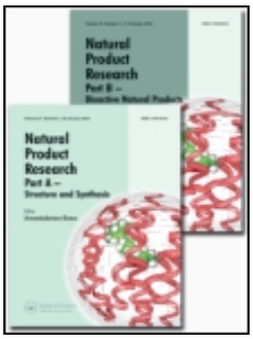

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# A new sesquiterpenoid from the rhizomes of *Homalomena sagittifolia*

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A new sesquiterpenoid, 1$\alpha$,4$\beta$,7$\beta$-eudesmanetriol (**1**), was isolated together with the known compounds 1$\beta$,4$\beta$,7$\beta$-eudesmanetriol (**2**) and oplopanone (**3**) from the rhizomes of *Homalomena sagittifolia*. The structures of these compounds were determined by extensive spectral analyses. The compounds **1** and **2** inhibited growth of *Pseudomonas stutzeri* with a MIC value of 117 μM when evaluated for antibacterial activity using the minimum concentration assay. Both these compounds showed remarkable activities against acetylcholinesterase enzyme with $IC_{50}$ values ranging between 25 and 26 μM. The isolation of these sesquiterpenoids and their biological activities observed in this study support the reported traditional uses of *H. sagittifolia* for the treatment of microbial related diseases and central nervous system disorders.

**Keywords:** *Homalomena sagittifolia*; Araceae; sesquiterpenoids; antibacterial activity; acetylcholinesterase

## 1. Introduction

*Homalomena* is a genus within the family Araceae comprising approximately 80–150 species found primarily in southern Asia and the southwestern Pacific (Bown, 2000). *H. sagittifolia* Jungh. is a 30–60 cm tall herb with a stout stem and dark green ovately arrow-shaped leaves found growing in damp, wet and low spaces in Malaysian forests. Here, a decoction of the roots and leaves is used as folk medicine to treat fever, while the pounded shoots are applied to distended stomachs (Anderson et al., 2003; Burkill, 1966; Hanum & Hamzah, 1999). Several *Homalomena* species have long been used in Asian traditional medicine for treating wounds, diarrhoea, fever, cough, colic, stomach disorders and certain types of central nervous system disorder (Schultes, 1984; Sung, Steffan, Steglich, Klebe, & Adam, 1992; Duke, 1985; Yong et al., 2008). A number of them were also reported to be hallucinogenic due to their

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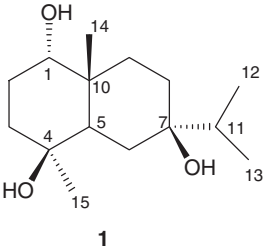
narcotic effects. Natives of Papua eat the leaves of *Homalomena* species with the bark of other plants to treat certain types of central nervous system disorders. The active constituents, however, are still unknown (Schultes, 1976, 1984). Previous phytochemical studies had revealed the presence of oplopane, eudesmane and eremophilane-type sesquiterpenoids in the rhizomes of *H. aromatica* and *H. occulta* (Sung et al., 1992; Y.F. Wang, X.Y. Wang, Lai, Lu, & Luo, 2007; Yong et al., 2008). Regarding *H. sagittifolia*, there has been no published data of its phytochemicals apart from a report on the analysis of its essential oil (Wong, Lim, & Ali, 2006). This article reports the isolation and identification of three sesquiterpenoids, one of them new, from the rhizomes of *H. sagittifolia,* and the *in vitro* antibacterial and anticholinesterase activities of these compounds.

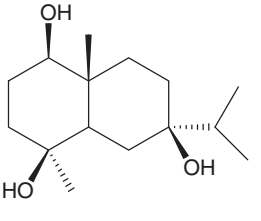
## 2. Results and discussion

### 2.1. *Identification of compound* 1

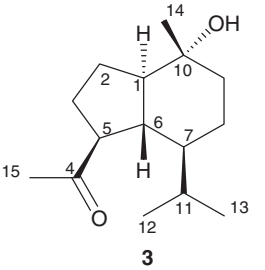
Compound **1** was obtained as a colourless amorphous solid. Its EIMS spectrum revealed no $M^+$ but fragment ion peaks at *m/z* 238 $[M\text{-}H_2O]^+$, 220 $[238\text{-}H_2O]^+$, 213 $[M\text{-}C_3H_7]^+$, 195 $[213\text{-}H_2O]^+$, 177 $[195\text{-}H_2O]^+$ and 159 $[177\text{-}H_2O]^+$, indicative of the presence of three hydroxyl groups. This was confirmed by the absorption bands in the IR spectrum at $\nu_{max}$ 3429 $cm^{-1}$, and $^{13}C$ NMR signals at $\delta$ 79.75, 70.94 and 73.30. The $^{13}C$ NMR and DEPT spectra revealed the presence of 15 carbons, comprising four methyl, five methylene, three methine and three quaternary carbons. The $^1H$ NMR spectrum indicated the presence of two tertiary methyl groups [$\delta$ 1.40 (3H, s) and $\delta$1.60 (3H, s)], and an isopropyl moiety [$\delta$ 1.07 (3H, d, $J = 6.8$ Hz) and $\delta$ 1.10 (3H, d, $J = 6.8$ Hz)]. In the $^1H$-$^1H$ COSY spectrum, the two methylene protons at $\delta$ 2.51 and 1.81 were seen to couple only with another two methylene protons at $\delta$ 1.64 and 1.89, and with the hydroxymethine proton at $\delta$ 3.68 which was assigned to C-1 ($\delta$ 79.75) based on HMBC correlation between the methyl protons H-14 ($\delta$ 1.60) with C-1. These spectral features were consistent with an eudesmane-type sesquiterpene skeleton (Adinarayana & Syamasundar, 1982).




**1**




**2**




**3**

Of the two remaining OH groups, one was assigned to the quaternary carbon C-4 ($\delta$ 70.94) based on HMBC correlation between the methyl protons H-15 ($\delta$1.40) with C-4, the other to the quaternary carbon C-7 based on HMBC correlations between the methyl protons H-12 ($\delta$ 1.07) and H-13 ($\delta$ 1.10) with C-7 ($\delta$ 73.30) and C-11 ($\delta$ 40.27) (Figure 1). The OH group at C-1 must occupy an axial position since the size of the coupling constants of H-1 (dd, $J = 4.0$ and 3.8 Hz) indicated that this proton is equatorial. The complete $^1H$ and $^{13}C$ NMR data obtained for **1** were

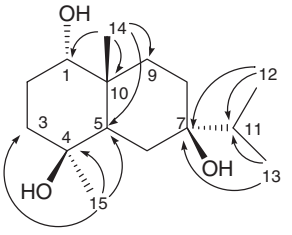



Figure 1. Pertinent HMBC correlations for **1**.

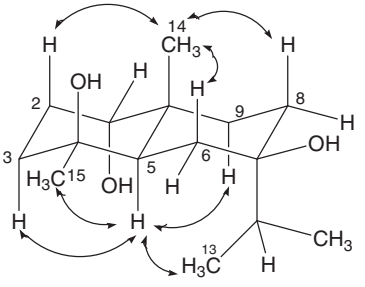



Figure 2. Selected NOESY correlations for **1**.

confirmed from results of extensive 2D NMR correlation measurements, including $^{1}$H-$^{1}$H COSY, HMQC and HMBC. The stereochemistry of **1** was determined by examining the NOESY spectrum which showed important correlations between H-14 ($\delta$ 1.60) with H-2$\beta$ ($\delta$ 1.81), H-6$\beta$ ($\delta$ 1.49) and H-8$\beta$ ($\delta$ 1.78); and between H-5 ($\delta$ 1.94) with H-3$\alpha$ ($\delta$ 1.89), H-15 ($\delta$ 1.40), H-9$\alpha$ ($\delta$ 2.31) and H-13 ($\delta$ 1.10) (Figure 2). Based on the above evidence, the structure of **1** was determined to be 1$\alpha$,4$\beta$,7$\beta$-eudesmanetriol, which has not been identified in a plant before, and whose spectral features were remarkably close to those of its epimer 1$\beta$, 4$\beta$, 7$\beta$-eudesmanetriol **2** isolated together in the present study and also previously from the rhizomes of *H. aromatica* (Sung et al., 1992) and from the roots of *H. occulta* (Wang et al., 2007).

### 2.2. *Antibacterial activity of* **1** *and* **2**

The minimum inhibitory concentration values (MICs) of the isolated sesquiterpenoids **1** and **2** as detected using the micro-dilution assay are shown in Table 1. These two compounds showed different levels of antibacterial activity with MIC values ranging between 117 and 390 μM against both Gram-positive and Gram-negative bacteria. MIC values recorded for tetracycline (positive control) ranged between 42–175 μM. Both **1** and **2** inhibited growth of *Pseudomonas stutzeri* (Gram-negative) with a MIC value of 117 μM, and possessed the same activity against *Escherichia coli* and *Klebsiella pneumoniae* (Gram-negative) with an MIC value of 234 μM.

The lack of understanding of the molecular mechanism of action of bioactive substances presents a major hindrance in the development of antibacterial agents. Several theories have been proposed for the mechanism of action of antibacterial

Table 1. Antibacterial activity of **1** and **2** as detected using the MIC assay.

| | MIC values (μM) | | | | |
|---|---|---|---|---|---|
| | Bacteria tested | | | | |
| Compounds | B.s | E.c | K.p | P.s | S.a |
| **1** | 117 ± 1.1 | 234 ± 2.5 | 234 ± 2.1 | 117 ± 1.4 | 234 ± 3.3 |
| **2** | 234 ± 1.9 | 234 ± 2.5 | 234 ± 2.3 | 117 ± 1.8 | 390 ± 4.1 |
| Tetracycline | 42 ± 0.4 | 175 ± 2.1 | 175 ± 1.6 | 87 ± 2.0 | 87 ± 1.1 |

Notes: Results obtained are presented as MIC values (μM) ± SD. B.s, *Bacillus subtilis* subsp. *spizizenii*; E.c, *Escherichia coli*; K.p, *Klebsiella pneumoniae*; P.s, *Pseudomonas stutzeri*; S.a, *Staphylococcus aureus*.

Table 2. Inhibition of acetylcholinesterase enzyme activity by **1** and **2**.

| Compounds | Inhibition of acetylcholinesterase ($IC_{50}$ μM) |
|---|---|
| **1** | 26 ± 4 |
| **2** | 25 ± 8 |
| Galanthamine | 3.3 ± 1.4 |

Note: Results are expressed as $IC_{50}$ values (μM) ± SD.

agents including drug interaction, alteration of target site and alteration and reduction of the metabolic pathway (Li & Nikaido, 2009). Interaction between bacterial cell and antibacterial agents may alter bacterial surface morphology leading to either increase or decrease of membrane permeability. Increase of permeability causes leakage of intracellular substances while decrease of permeability prevents nutrient transport (Freiberg, Fischer, & Brunner, 2005; Tang et al., 2010). This may explain in part the antibacterial effects observed by the tested compounds in the present study. Compounds **1** and **2** showed relatively weak activity against *Staphylococcus aureus* (Gram-positive) compared to *Bacillus subtilis* subsp. *spizizenii*. *S. aureus* is one of the major resistant pathogens. One of the major key works of antibiotics against *S. aureus* was based on $\beta$-lactam antibiotics. However, the clinical usefulness of these drugs is limited by their susceptibility to $\beta$-lactamase. A number of *S. aureus* are known to produce $\beta$-lactamase. This enzyme causes, among other factors, lack of efficiency of antibacterial agents against *S. aureus* (Esimone, Okoye, Nworu, & Agubata, 2008). This may justify the relatively weak activity of the two sesquiterpenoids against *S. aureus* observed in this study.

### 2.3. *Anti-cholinesterase activity of* 1 *and* 2

The cholinesterase inhibitory activity of the isolated compounds **1** and **2** obtained by using the microplate assay and indicated as $IC_{50}$ values are presented in Table 2.

Compounds showing inhibitory effects against acetylcholinesterase enzyme with $IC_{50} \leq 50\,\mu M$ were considered active. Both **1** and **2** showed remarkable activities against acetylcholinesterase with $IC_{50}$ values ranging between 25 and 26 μM. The $IC_{50}$ for the galanthamine (positive control) was 3.3 μM.

Acetylcholine is an organic molecule liberated at nerve endings as a neurotransmitter. It is produced by the synthetic enzyme choline acetyltransferase that uses acetyl co-enzyme A and choline as substrates for the formation of acetylcholine in specific cells known as cholinergic neurons. The principle role of acetylcholinesterase (AChE) is termination of impulse transmission at cholinergic synapses by rapid hydrolysis of acetylcholine (Bar-On et al., 2002). Owing to its essential biological role in the mediation of vertebrate and invertebrate nervous transmission, acetylcholinesterase has been medicinally targeted in treatments for Alzheimer's disease, myasthenia gravis, glaucoma, pesticides and snake venom toxins (Eldeen, Van Staden, & Elgorashi, 2010; Ion et al., 2010).

Acetylcholinesterase inhibitor drugs are effective in delaying neurocognitive decline in people with mild to moderate severity of Alzheimer's disease. Both the sesquiterpenoids tested in this study showed activity against acetylcholinesterase enzyme. This finding supports the uses of the plant for the traditional treatment of diseases related to central nervous system. Sesquiterpenes and other terpenes are known to be of deterrent effects against chemosensory detection systems in target organisms (Escalera, von Hehn, Bessac, Sivula, & Jordt, 2008). This biological activity may be in part due to the ability of these compounds to inhibit acetylcholinesterase enzyme activity as was observed by the sesquiterpenes **1** and **2** in this study. The observed biological activities in this study represent a scientific base for evaluation and formulation of the traditional uses of *H. sagittifolia* as antibacterial agents and for the traditional treatment of some disorders of the central nervous system.

## 3. Experimental

### 3.1. *General*

Melting points were determined using a Melting Point SMP1 apparatus (Stuart Scientific) and are uncorrected. Analytical and preparative TLC was carried out on Merck 60 $GF_{254}$ silica gel plates (absorbent thickness: 0.25 and 0.50 mm, respectively). CC was performed using silica gel (Merck 230–400 mesh, ASTM). NMR spectra were recorded in either pyridine-$d_5$ or $CDCl_3$ with tetramethylsilane as an internal standard, using a Bruker Avance 400 and a Bruker Avance 300 spectrometer. IR spectra were recorded on a Perkin–Elmer System 2000 FTIR instrument. EIMS were recorded on a Finnigan MAT 95 operating at 70 eV. Optical rotations were obtained using a JASCO DIP-370 digital polarimeter.

### 3.2. *Plant material*

*H. sagittifolia* was collected from Redang Panjang, Selama, Perak in December 2005 and identified by one of the authors (S. Baharuddin). A voucher specimen (USM 10126) has been deposited in the Herbarium of Universiti Sains Malaysia in Penang.

### 3.3. *Extraction and isolation*

Air-dried and powdered rhizomes (200 g) were macerated at room temperature with aqueous methanol (2 l × 3) for 14 h. The combined filtrate was concentrated under reduced pressure at 50°C to yield a syrupy residue (23 g) which was extracted with $CHCl_3$ (200 mL × 3) to give 0.9 g of a dark brown gum. The $CHCl_3$ extract was fractionated on a silica gel column, eluting with hexane-EtOAc (7 : 3 → 1 : 4 v/v) to give fractions Fr. 1-7. Fr. 2, which eluted with hexane-EtOAc (7 : 3 → 2 : 1 v/v), was rechromatographed on a silica gel column using a hexane-EtOAc gradient to afford **3** (9 mg) after purification by PTLC using $CHCl_3$ as the solvent. Fr. 3, which eluted with hexane-EtOAc (2 : 1 → 5 : 3 v/v), was rechromatographed on a silica gel column by elution with a hexane-EtOAc gradient to yield **1** (7 mg) after purification by PTLC eluting with $CHCl_3$-MeOH (9 : 1 v/v). Fr. 4, which eluted with hexane-EtOAc (2 : 3 v/v), was further fractionated on a silica gel column, eluting with $CHCl_3$, to give **2** (6 mg) after purification by PTLC using 12 % MeOH in $CHCl_3$.

*1α, 4β, 7β-eudesmanetriol* (**1**): Colourless solid, m.p. 128–129 °C; $[\alpha]_D^{30}$ –78 ($CHCl_3$; c 3.5); IR (KBr): $\nu_{max}$ = 3429, 3017, 2927, 2861, 1608, 1493, 1452, 1403 $cm^{-1}$; EIMS 70 eV, *m/z* (rel. int.) = 238 ($[M\text{-}H_2O]^+$, 2.0), 220 ($[238\text{-}H_2O]^+$, 2.3), 213 ($[M\text{-}C_3H_7]^+$, 21.5), 195 ($[213\text{-}H_2O]^+$, 100), 177 ($[195\text{-}H_2O]^+$, 47.8) and 159 ($[177\text{-}H_2O]^+$, 28.1); $^1H$ NMR (400 MHz, pyridine-$d_5$): δ 1.07 (3H, d, *J* = 6.8 Hz, H-12), 1.10 (3H, d, *J* = 6.8 Hz, H-13), 1.40 (3H, s, H-15), 1.49 (1H, m, H-6β), 1.60 (3H, s, H-14), 1.64 (1H, m, H-3β), 1.74 (1H, m, H-8α), 1.78 (2H, m, H-8β, H-11), 1.81 (1H, m, H-2β), 1.89 (1H, m, H-3α), 1.92 (1H, m, H-9β), 1.94 (1H, m, H-5), 2.07 (1H, m, H-6α), 2.31 (1H, m, H-9α), 2.51 (1H, m, H-2α) and 3.68 (1H, dd, *J* = 4.0, 3.8 Hz, H-1); $^{13}C$ NMR (100 Mz, pyridine-$d_5$): δ 79.75 (C-1), 28.49 (C-2), 41.20 (C-3), 70.94 (C-4), 45.97 (C-5), 30.84 (C-6), 73.30 (C-7), 29.84 (C-8), 35.84 (C-9), 40.24 (C-10), 40.27 (C-11), 17.64 (C-12), 17.77 (C-13), 12.76 (C-14) and 30.63 (C-15).

*1β, 4β, 7β-eudesmanetriol* (**2**): Colourless solid, m.p. 120–121 °C; $[\alpha]_D^{30}$ – 48 ($CHCl_3$; c 3.0); IR (KBr): $\nu_{max}$ = 3402, 2959, 2929, 2870, 1458, 1376, 1306, 1262 $cm^{-1}$; EIMS 70 eV, *m/z* (rel. int.) = 238 ($[M\text{-}H_2O]^+$, 2.0), 223 ($[238\text{-}CH_3]^+$, 1.0), 213 ($[M\text{-}C_3H_7]^+$, 19.5), 195 ($[213\text{-}H_2O]^+$, 90.0), 177 (38.8) and 43 (100); $^1H$ NMR (400 MHz, $CDCl_3$): δ 0.95 (3H, d, *J* = 6.9 Hz, H-12), 0.97 (3H, d, *J* = 6.9 Hz, H-13), 1.03 (3H, s, H-14), 1.17 (3H, s, H-15), 1.42 (1H, m, H-9β), 1.46 (1H, m, H-6β), 1.50 (1H, m, H-5), 1.58 (1H, m, H-8β), 1.60 (1H, m, H-3β), 1.62 (1H, m, H-6α), 1.63 (1H, m, H-11), 1.65 (1H, m, H-2β), 1.68 (1H, m, H-8α), 1.69 (1H, m, H-9α), 1.71 (1H, m, H-3α), 1.90 (1H, m, H-2α) and 3.33 (1H, dd, *J* = 9.9, 3.8 Hz, H-1); $^{13}C$ NMR (100 Mz, $CDCl_3$): δ 79.76 (C-1), 27.15 (C-2), 40.09 (C-3), 71.99 (C-4), 45.02 (C-5), 29.68 (C-6), 74.16 (C-7), 29.40 (C-8), 34.99 (C-9), 39.25 (C-10), 39.57 (C-11), 17.19 (C-12), 17.34 (C-13), 11.97 (C-14) and 30.23 (C-15). The above spectroscopic data were in good agreement with published data (Sung et al., 1992; Wang et al., 2007).

*Oplopanone* (**3**): Colourless solid, m.p. 88–89 °C; $[\alpha]_D^{28}$ –22 ($CHCl_3$; c 4.0); IR (KBr): $\nu_{max}$ = 3408, 2956, 2934, 2872, 1709, 1456, 1386, 1368, 1332, 1128 $cm^{-1}$; EIMS 70 eV, *m/z* (rel. int.) = 238 ($M^+$, 4.4), 220 ($[M\text{-}H_2O]^+$, 0.7), 177 (5.7), 153 (100), 135 (76.5), 111 (19.5), 71 (24.6) and 43 (98.0); $^1H$ NMR (400 MHz, $CDCl_3$): δ 0.70 (3H, d, *J* = 6.9 Hz, H-13), 0.90 (3H, d, *J* = 6.9 Hz, H-12), 1.08 (1H, m, H-7), 1.10 (1H, m, H-8a), 1.20 (3H, s, H-14), 1.38 (1H, m, H-9a), 1.42 (1H, m, H-2a), 1.45 (2H, m, H-1, H-11), 1.58 (1H, m, H-3a), 1.60 (1H, m, H-8b), 1.80 (1H, m, H-9b), 1.82 (1H, m,

H-6), 1.83 (1H, m, H-2b), 1.97 (1H, m, H-3b), 2.16 (3H, s, H-15), 2.63 (1H, ddd and $J = 10.2$, 5.5, 5.4 Hz, H-5); $^{13}$C NMR (100 Mz, $CDCl_3$): $\delta$ 57.4 (C-1), 25.7 (C-2), 28.9 (C-3), 211.8 (C-4), 56.1 (C-5), 47.1 (C-6), 49.8 (C-7), 23.4 (C-8), 42.4 (C-9), 73.4 (C-10), 29.9 (C-11), 22.3 (C-12), 16.0 (C-13), 20.7 (C-14) and 29.8 (C-15). The above spectroscopic data were in good agreement with published data (Jung et al., 1997).

### 3.4. *Micro-dilution antibacterial assay*

The serial dilution technique by Eloff (1998), using 96-well micro-plates, was employed to determine the MIC of the isolated compounds **1** and **2**. Two millilitre cultures of two Gram-positive bacterial strains, *Bacillus subtilis* subsp. *spizizenii* (ATCC6633) and *Staphylococcus aureus* (ATCC12600), and three Gram-negative bacterial strains, *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* (ATCC13883) and *Pseudomonas stutzeri* (ATCC17588), were prepared and placed in an incubator overnight at 37°C. The overnight cultures were diluted with sterile Mueller–Hinton (MH) broth (1 mL bacteria / 50 mL MH) to yield a density of bacterial cells between $10^6$ and $10^8$ cells per mL. The tested compounds were resuspended to a concentration of 10 mg mL$^{-1}$ with ethanol to yield a final concentration of 2.5 mg mL$^{-1}$ in the assay. For each of the five bacteria used, 100 µl of the test solution were serially diluted two-fold with 100 µl of sterile distilled water in a sterile 96-well micro-plate. A similar two-fold serial dilution of tetracycline (1 mg mL$^{-1}$) was used as a positive control against each bacterium. One hundred µl of each bacterial culture were added to each well. The plates were covered and incubated overnight at 37°C. To indicate bacterial growth, 50 µl of 0.2 mg mL$^{-1}$ *p*-iodonitrotetrazolium violet (INT) was added to each well, and the plates were incubated at 37°C for 30 min. Bacterial growth in the wells was indicated by a red colour, whereas clear wells indicated inhibition by the tested substances.

### 3.5. *Acetylcholinesterase enzyme inhibitory activity*

Inhibition of acetylcholinesterase by the isolated compounds **1** and **2** was investigated using the microplate assay. The assay is based on Ellman's method (Ellman, Courtney, Andres, & Featherstone, 1961) with modifications (Rhee, van de Meent, Ingkaninan, & Verpoorte, 2001; Rhee, Van Rijn, & Verpoorte, 2003). The enzyme activity is measured by observing the increase of a yellow colour produced from thiocholine when it reacts with the dithiobisnitrobenzoate ion. Acetylthiocholine iodide (ATCl), acetylcholinesterase (AChE) from electric eels (type VI-S lypophilised powder), 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and galanthamine were obtained from Sigma-Aldrich. The following buffers were used; Buffer A: 50 mM Tris-HCl, pH 8; Buffer B: 50 mM Tris-HCl, pH 8 containing 0.1% bovine serum albumin (BSA); Buffer C: 50 mM Tris-HCl, pH 8, containing 0.1 M NaCl and 0.02 M $MgCl_2.6H_2O$. The assay was performed using an Opsys MR 96-well microplate reader. In the 96-well plates, 25 µL of 15 mM ATCl in water, 125 µL of 3 mM DTNB in buffer C, 50 µl of buffer B and 25 µl of a serially diluted (two fold) solution of the isolated compounds (initial concentration of 60 µM) and galanthamine hydrobromide as positive control (intitial concentration of 20 µM) were added. The absorbance was measured at 405 nm every 45 sec (five times).

Then 25 µl of 0.2 U $mL^{-1}$ solution of enzyme were added, the absorbance was measured again every 45 sec (eight times). The rate of reaction was calculated. Any increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the ratio of reaction before adding the enzyme from the rate after adding the enzyme. Percentage inhibition (eliminated) was calculated by comparing the reaction rates for the sample to the blank (methanol in buffer A). The assay was performed in triplicate. $IC_{50}$ values were calculated using a logarithmic non-linear regression curve derived from the plotted data using GraphPad Prism software version 4.03 (GraphPad Software, Inc., San Diego, USA).

## 4. Conclusions

The phytochemical investigation of rhizomes of *H. sagittifolia* revealed the presence of oplopane and eudesmane-type sesquiterpenoids which seem to be common in species of the genus *Homalomena*. One of the compounds isolated, (−)-1$\alpha$, 4$\beta$, 7$\beta$-eudesmanetriol (**1**), has, to our knowledge, not been reported from a plant before. This sesquiterpenoid and its C-1 epimer (**2**) were found to exhibit remarkable activities against acetylcholinesterase enzyme thus lending credence to the reported traditional uses of *H. sagittifolia* for the treatment of central nervous system disorders.

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