



## Anticholinesterase and Anti-inflammatory Constituents from *Beilschmiedia pulverulenta* Kosterm

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**Abstract** – Phytochemical investigation from the stem bark of *Beilschmiedia pulverulenta* resulted in the isolation of five lignans, (+)-yangambin (**1**), (+)-sesartemin (**2**), (+)-excelsin (**3**), (+)-sesamin (**4**), and (+)-syringaresinol (**5**), together with lupeol (**6**), lupenone (**7**),  $\beta$ -sitosterol (**8**), and  $\beta$ -sitostenone (**9**). Their structures were established by the analysis of their spectroscopic (1D and 2D NMR) and spectrometric (MS) data, as well as by comparison with those reported in the literature. The isolated lignans were tested for their anticholinesterase (AChE: acetylcholine esterase and BChE: butyryl cholineesterase) and anti-inflammatory (COX-2: cyclooxygenase-2 and LOX: lipoxygenase) activities. All the isolated lignans (**1** – **5**) exhibited significant inhibition activities in AChE/BChE and COX-2/LOX assays with IC<sub>50</sub> values ranging from 168.8 – 504.2  $\mu$ M and 21.0 – 59.4  $\mu$ M, respectively.

**Keywords** – Anticholinesterase, Anti-inflammatory, Lignan, *Beilschmiedia pulverulenta*

### Introduction

Alzheimer disease (AD) is an irreversible neurodegenerative disorder which effects memory loss, learning disabilities, cognitive impairments, and diverse range of neuropsychiatric symptoms.<sup>1</sup> Acetylcholinesterase (AChE) has proven to be the most viable therapeutic target for symptomatic improvement in AD. Besides AChE which is found primarily in the blood and neural synapses is butyrylcholinesterase (BChE), an enzyme found in the liver. Many of drugs available for treatment of AD target both AChE and BChE but some are more selective than others.<sup>2</sup> The role of anti-inflammatory agents in the prevention or treatment of AD has been the focus of current research. According to Borovikova *et al.*,<sup>3</sup> there is an established link between the cholinergic system and inflammation, with acetylcholine playing a role in cytokine release. In addition, Tabet<sup>4</sup> reported on evidence that acetylcholinesterase inhibitors have an anti-inflammatory role by indirectly increasing the production of antioxidants in the brain, thereby acting against free radicals, amyloid

toxicity and a reduction in release of cytokines from activated microglia in the brain and blood.

The genus *Beilschmiedia* comprises nearly 250 species and is abundant in Africa and Asia. Previous phytochemical investigations have shown that several classes of natural products including, endiandric acid derivatives, alkaloids, flavonoids, terpenoids, lignans, neolignans and essential oils.<sup>5,6,7</sup> A few of these compounds are reported to exhibit antibacterial, antimalarial and anti-tuberculosis activities.<sup>8,9</sup> *Beilschmiedia pulverulenta* is locally known as ‘*medang merah*’ in Malaysia and distributed in the Peninsular Malaysia, Borneo and Indonesia. It grows on sandy loam soils in mixed dipterocarp forest.<sup>10</sup> The chemical compositions and biological activities of the essential oil from *B. pulverulenta* have been reported by us.<sup>11</sup> Herein, we would like to report the chemical constituents from the stem bark of *B. pulverulenta* together with their anticholinesterase and anti-inflammatory activities. To the best of our knowledge, there is no report on the constituents and bioactivity studies from this species.

### Experimental

**General experimental procedures** – The optical rotations were recorded on a Perkin Elmer 341 Polarimeter

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equipped with a sodium lamp and chloroform as solvent. The mass spectra were recorded on Bruker Mass Spectrometry Services, obtained from National University of Singapore (NUS). The UV spectra were obtained in methanol on a Shimadzu UV 1601PC spectrophotometer. The IR spectra were obtained on a Perkin-Elmer 1600 FTIR spectrophotometer. The  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra were recorded in deuterated chloroform on a Bruker Avance 400 MHz spectrometer; chemical shifts were reported in ppm on  $\delta$  scale, and the coupling constants were given in Hz. Column chromatography was carried out on silica gel 70 - 230 mesh (Merck). Silica gel 60 F<sub>254</sub> precoated aluminium plates (0.2 mm, Merck) were used for TLC analysis. The TLC and PTLC spots were visualized under UV light (254 and 366 nm) followed by spraying with 5% H<sub>2</sub>SO<sub>4</sub> in methanol and 1% vanillin in MeOH, followed by heating at 120 °C for 5 min. All solvents were AR grade.

**Plant materials** – A sample of *B. pulverulenta* was collected from mixed dipterocarp forests located in Kuching and Samarahan Districts of Sarawak in January 2010 and identified by Mohizar Mohamad. The voucher specimens (UiTMKS 4014) were deposited at the Natural Product Research and Development Centre (NPRDC), Universiti Teknologi MARA Sarawak.

**Extraction and isolation** – Cold extraction of the powdered stem bark (300 g) with *n*-hexane, EtOAc and MeOH yielded the crude stem bark of *n*-hexane (BPBH: 5.02 g), EtOAc (BPBE: 8.78 g) and MeOH (BPBM: 7.29 g) extracts. The *n*-hexane extract was fractionated by vacuum liquid chromatography (VLC) and eluted with *n*-hexane:EtOAc:MeOH to give six fractions (BPBE1-6). Fractions BPBE5-6 were subjected to column chromatography (CC) over silica gel (800 g, 12×100 cm) eluted with *n*-hexane:CHCl<sub>3</sub> (90:10, 80:20, 75:25, 70:30, 60:40, 50:50, 45:55, 40:60, 35:65, 30:70) to afford 150 fractions. Fraction 90-130 was recrystallized from cold *n*-hexane to give (1) (50.1 mg: white solid) and (2) (25.2 mg: white solid). Fractions BPBE1-4 were subjected to CC over silica gel (600 g, 5×60 cm) eluted with *n*-hexane:EtOAc (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50) to afford 100 fractions. Fraction 20-35 and 40-50 was recrystallized from cold *n*-hexane to yield (6) (5.2 mg: white needle) and (7) (12.9 mg: colourless needle), respectively. The EtOAc extract was fractionated by VLC and eluted with *n*-hexane:EtOAc:MeOH to give five fractions (BPBE1-5). Fractions BPBE3-5 were subjected to CC over silica gel (600 g, 5×60 cm) eluted with *n*-hexane:CHCl<sub>3</sub> (90:10, 80:20, 70:30, 60:40, 50:50) to afford 200 fraction. Fraction 60-130 was subjected to CC

over silica gel (300 g, 5×60 cm) eluted with *n*-hexane:EtOAc to afford 100 fractions. Fraction 55 - 65 and 70 - 90 recrystallized from cold *n*-hexane to yield (4) (10.5 mg: colourless needle) and (5) (15.9 mg: colourless rhombic crystal), respectively. Fractions BPBE1-2 were subjected to CC over silica gel (500 g, 5×60 cm) eluted with *n*-hexane:CHCl<sub>3</sub> (90:10, 80:20, 75:25, 70:30, 60:40, 50:50) to afford 100 fractions. Fraction 20-35 was recrystallized from cold *n*-hexane and yielded (8) (18.2 mg: white crystalline needles) and (9) (21.4 mg: white solid). The MeOH extract was fractionated by VLC and eluted with *n*-hexane:EtOAc:MeOH to give five fractions (BPBE1-5). Fractions BPBE3-5 was subjected to CC over silica gel (200 g, 5×60 cm) eluted with *n*-hexane:CHCl<sub>3</sub> (90:10, 80:20, 75:25, 70:30, 60:40, 50:50) to afford 100 fractions. Fraction 50 - 80 were combined and followed by preparative TLC (*n*-hexane:EtOAc, 1:1) to yield (3) (10.5 mg: white solid).

**(+)-Yangambin (1)** – White solid.  $[\alpha]_{\text{D}}^{25}$ : +85.5° (*c* 0.02, CHCl<sub>3</sub>); MP: 125 - 128 °C; IR (KBr)  $\nu_{\text{max}}$ : 3076, 2952, 1590, 1511, 1237 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{\text{max}}$  nm: 257 and 271;  $^1\text{H NMR}$  (400 MHz, CDCl<sub>3</sub>):  $\delta$  3.12 (2H, m, H-8/H-8'), 3.86 (6H, s, 2×OCH<sub>3</sub>), 3.89 (12H, s, 4×OCH<sub>3</sub>), 3.96 (2H, dd, *J* = 9.2, 3.6 Hz, H-9 $\beta$ /H-9 $\beta'$ ), 4.34 (2H, dd, *J* = 9.2, 3.6 Hz, H-9 $\alpha$ /H-9 $\alpha'$ ), 4.76 (2H, d, *J* = 4.4 Hz, H-7/H-7'), 6.59 (4H, s H-2/H-6/H-2'/H-6');  $^{13}\text{C NMR}$  (100 MHz, CDCl<sub>3</sub>):  $\delta$  54.3 (C-8/C-8'), 56.2 (4×OCH<sub>3</sub>), 60.8 (2×OCH<sub>3</sub>), 71.9 (C-9/C-9'), 85.9 (C-7/C-7'), 102.9 (C-2/C-2'/C-6/C-6'), 136.7 (C-1/C-1'), 137.6 (C-4/C-4'), 153.4 (C-3/C-3'/C-5/C-5'); EIMS: *m/z* 446 [M<sup>+</sup>, C<sub>24</sub>H<sub>30</sub>O<sub>8</sub>].<sup>12</sup>

**(+)-Sesartemin (2)** – White solid.  $[\alpha]_{\text{D}}^{25}$ : +42.6° (*c* 0.02, CHCl<sub>3</sub>); MP: 110 - 112 °C; IR (KBr)  $\nu_{\text{max}}$ : 3071, 2952, 1587, 1511, 1237 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{\text{max}}$  nm: 257 and 271;  $^1\text{H NMR}$  (400 MHz, CDCl<sub>3</sub>):  $\delta$  3.09 (2H, m, H-8/H-8'), 3.85 (3H, s, 4'-OCH<sub>3</sub>), 3.89 (6H, s, 3'/5'-OCH<sub>3</sub>), 3.93 (3H, s, 5-OCH<sub>3</sub>), 3.93 (4H, m, H-9 $\beta$ /H-9 $\beta'$ -overlapping), 4.31 (4H, m, H-9 $\alpha$ /H-9 $\alpha'$ ), 4.74 (2H, d, *J* = 2.8 Hz, H7/H-7'), 5.98 (2H, s, OCH<sub>2</sub>O), 6.54 (1H, d, *J* = 1.5 Hz, H-2), 6.56 (1H, d, *J* = 1.5 Hz, H-6), 6.58 (2H, s, H2/H-6');  $^{13}\text{C NMR}$  (100 MHz, CDCl<sub>3</sub>):  $\delta$  54.3 (C-8'), 54.3 (C-8), 56.2 (3'/5'-OCH<sub>3</sub>), 56.7 (5-OCH<sub>3</sub>), 60.8 (4'-OCH<sub>3</sub>), 71.8 (C-9), 71.9 (C-9'), 85.8 (C-7'), 85.9 (C-7), 100.0 (C-2), 101.4 (OCH<sub>2</sub>O), 102.9 (C-2'/C-6'), 105.7 (C-6), 134.6 (C-4), 135.8 (C-1), 136.7 (C-1'), 137.5 (C-4'), 143.6 (C-5), 149.1 (C-3), 153.4 (C-3'/C-5'); EIMS: *m/z* 430 [M<sup>+</sup>, C<sub>23</sub>H<sub>26</sub>O<sub>8</sub>].<sup>12</sup>

**(+)-Excelsin (3)** – White solid.  $[\alpha]_{\text{D}}^{25}$ : +124.9° (*c* 0.02, CHCl<sub>3</sub>); MP: 109 - 112 °C; IR (KBr)  $\nu_{\text{max}}$ : 3071, 2953, 1587, 1510, 1237 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{\text{max}}$  nm: 259 and 275;  $^1\text{H NMR}$  (400 MHz, CDCl<sub>3</sub>):  $\delta$  3.06 (2H, m, H-8/H-8'), 3.88 (2H, m, H-9 $\beta$ /9 $\beta'$ ), 3.93 (6H, s, 2×OCH<sub>3</sub>), 4.27

(2H, m, H-9 $\alpha$ /9 $\alpha'$ ), 4.72 (2H, d,  $J$  = 4.0 Hz, H-7/H-7'), 6.98 (2H, s, 2 $\times$ OCH<sub>2</sub>O), 6.54 (2H, d,  $J$  = 0.8 Hz, H-2/H-2'), 6.56 (2H, d,  $J$  = 0.8 Hz, H-6/H-6'); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  54.3 (C-8/C-8'), 56.7 (2 $\times$ OCH<sub>3</sub>), 71.8 (C-9/C-9'), 85.8 (C-7/C-7'), 102.0 (2 $\times$ OCH<sub>2</sub>O), 101.4 (C-2/C-2'), 105.6 (C-6/C-6'), 134.6 (C-4/C-4'), 135.8 (C-1/C-1'), 143.6 (C-5/C-5'), 149.1 (C-3/C-3'); EIMS:  $m/z$  414 [M<sup>+</sup>, C<sub>22</sub>H<sub>22</sub>O<sub>8</sub>].<sup>13</sup>

**(+)-Sesamin (4)** – Colourless needle. [ $\alpha$ ]<sub>D</sub><sup>25</sup>: +30.6° ( $c$  0.01, CHCl<sub>3</sub>); MP: 117 - 119 °C; IR (KBr)  $\nu_{\max}$ : 2851, 1500, 1443, 1251 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{\max}$  nm: 257 and 287; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  3.07 (1H, m, H8/8'), 3.90 (2H, dd,  $J$  = 9.2, 3.6 Hz, H-9 $\alpha$ /9 $\alpha'$ ), 4.26 (2H, dd,  $J$  = 9.2, 6.8 Hz, H-9 $\beta$ /9 $\beta'$ ), 4.74 (1H, d,  $J$  = 4.4 Hz, H-7/7'), 5.97 (2H, s, OCH<sub>2</sub>O), 6.79 (1H, d,  $J$  = 8.0 Hz, H-5/5'), 6.82 (1H, d,  $J$  = 8.0 Hz, H-6/6'), 6.87 (1H, s, H-2/2'); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  54.3 (C-8/8'), 71.7 (C-9/9'), 85.8 (C-7/7'), 101.0 (2 $\times$ OCH<sub>2</sub>O), 106.4 (C-5/5'), 108.1 (C-6/6'), 119.3 (C-2/2'), 135.0 (C-4/4'), 147.1 (C-3/3'), 147.9 (C-1/1'); EIMS:  $m/z$  354 [M<sup>+</sup>, C<sub>29</sub>H<sub>50</sub>O].<sup>14</sup>

**(+)-Syringaresinol (5)** – Colourless needle. [ $\alpha$ ]<sub>D</sub><sup>25</sup>: +143.7° ( $c$  0.02, CHCl<sub>3</sub>); MP: 183 - 184 °C; IR (KBr)  $\nu_{\max}$ : 2851, 1500, 1443, 1251 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{\max}$  nm: 259 and 281; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  3.11 (1H, m, H8/8'), 3.90 (12H, s, 4 $\times$ OCH<sub>3</sub>), 3.94 (2H, m, H-9 $\beta$ /9 $\beta'$ ), 4.30 (2H, m, H-9 $\alpha$ /9 $\alpha'$ ), 4.75 (1H, d,  $J$  = 4.4 Hz, H-7/7'), 5.9756 (2H, s, 2 $\times$ OH), 6.60 (4H, s, H-2/2'/6/6'); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  54.3 (C-8/8'), 56.3 (4 $\times$ OCH<sub>3</sub>), 71.8 (C-9/9'), 86.0 (C-7/7'), 102.7 (C-2/6/2'/6'), 132.1 (C-1/1'), 134.3 (C-4/4'), 147.1 (C-3/5/3'/5'); EIMS:  $m/z$  418 [M<sup>+</sup>, C<sub>22</sub>H<sub>26</sub>O<sub>8</sub>].<sup>14</sup>

**Lupeol (6)** – White needles. MP: 204 - 206 °C; IR (KBr)  $\nu_{\max}$ : 3434, 2927, 1634, and 1070 cm<sup>-1</sup>; spectral data were consistent with the literature.<sup>15</sup>

**Lupenone (7)** – Colourless needle. MP: 169 - 171 °C; IR (KBr)  $\nu_{\max}$ : 3068, 2934, 1704, 1453, and 1380 cm<sup>-1</sup>; spectral data were consistent with the literature.<sup>16</sup>

**$\beta$ -Sitosterol (8)** – White crystalline needles. MP: 133 - 134 °C; IR (KBr)  $\nu_{\max}$ : 3435, 2966, 1461, and 1376 cm<sup>-1</sup>; spectral data were consistent with the literature.<sup>17</sup>

**$\beta$ -Sitostenone (9)** – White solids; MP: 77 - 79 °C; IR (KBr)  $\nu_{\max}$ : 3444, 2960, 1687, 1460, 1414, and 1222 cm<sup>-1</sup>; spectral data were consistent with the literature.<sup>17</sup>

**Anticholinesterase activity** – AChE/BChE inhibitory activity of the lignans was measured by slightly modifying the spectrophotometric method.<sup>18,19</sup> Electric eel AChE and horse serum BChE were used, while acetylthiocholine iodide and butyrylthiocholine chloride were employed as substrates of the reaction. 5,5'-Dithio-bis(2-nitrobenzoic acid (DTNB) was used for the measurement of the

anticholinesterase activity. Briefly, 140  $\mu$ L of sodium phosphate buffer (pH 8.0), 20  $\mu$ L of DTNB, 20  $\mu$ L of the test samples and 20  $\mu$ L of AChE/BChE solution were added by multichannel automatic pipette to a 96-well microplate and incubated for 15 min at 25 °C. The reaction was then initiated by the addition of 10  $\mu$ L of acetylthiocholine iodide/butyrylthiocholine chloride. Hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at 412 nm utilizing a 96-well microplate reader (Epoch Micro-Volume Spectrophotometer, USA). Percentage of inhibition (%) of AChE/BChE was determined by comparison of the rate of reaction of each sample relative to a blank sample (ethanol in phosphate buffer pH = 8) using the following formula:

$$\% = [E - S / E] \times 100$$

where E is the activity of enzyme without the test sample and S is the activity of enzyme with test sample. Galantamine was used as the standard. The experiments are reported as mean  $\pm$  SD of triplicates.

#### Anti-inflammatory activity

**Cyclooxygenase-2 (COX-2) assay** – The anti-inflammatory activity of the compounds on COX-2 was determined by measuring prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) using a COX Inhibitor Screening Kit (Catalog No 560131, Cayman Chemicals, Ann Arbor Michigan USA) as well as the reported method.<sup>16</sup> Reaction mixtures containing the reaction buffer (950  $\mu$ L), heme (10  $\mu$ L), COX-2 (10  $\mu$ L) and sample/control (20  $\mu$ L) were incubated at 37 °C in a water bath for 15 min. The reaction was initiated by addition of arachidonic acid (10  $\mu$ L) at a final concentration of 100  $\mu$ M. After 2 min incubation, the reactions were stopped by addition of 1 M HCl (50  $\mu$ L), followed by saturated solution of stannous chloride (100  $\mu$ L). Then, prostaglandins (PG) were quantified by means of the ELISA method. The contents of the reaction tubes were diluted and transferred to a 96-well plate coated with a mouse antirabbit IgG, followed by addition of the PG screening acetylcholinesterase tracer and the PG screening antiserum. Plates were incubated in an orbital shaker for 18 h, at room temperature. The reaction mixtures were removed, and the wells were washed five times with a buffer containing 0.05% Tween 20. Acetylthiocholine and 5,5'-dithio-bis-2-nitrobenzoic acid known as Ellman's reagent (200  $\mu$ L) was then added to each well, and the plate was incubated in an orbital shaker for 60 min, at room temperature, until the control wells yielded an

optical density lying between 0.3 - 0.8 at 415 nm. A standard curve with PG was generated from the same plate, which was used to quantify the PG levels produced in the presence of the samples. The compound DuP697 (Cayman Chemicals) was used to standardize the assay for COX-2 and indomethacin was employed as positive control. Results were expressed as a percentage relative to a control (100% initial activity, solvent treated samples). All determinations were performed in triplicate. Regression analysis was employed for the calculation of IC<sub>50</sub> values.

**Lipoxygenase (LOX) assay** – The reagents were prepared according to the standard protocol (Lipoxygenase inhibitor screening assay kit, Item No. 760700 Cayman Chemicals Co). Stock solutions of compounds were prepared so as to obtain concentrations of 100 - 6.25 μM in the respective wells. The prepared solutions were then introduced onto 96 well plates where the cells were distributed as blanks 1A-2A-1D (triplicate), positive control 1B-2B (duplicate), and 100% initial activity wells 1C-2C-2D (triplicate). The remaining wells were designated for inhibitor (tested sample) solutions in duplicate. The addition of the reagents was done according to the standard protocol, according to which, 100 μL of assay buffer was added to the blank wells and 90 μL of lipoxygenase (5-LOX) enzyme and 10 μL of assay buffer were added to positive control wells. For the 100% initial activity wells, 90 μL of lipoxygenase enzyme and 10 μL of solvent (DMSO) were added. The inhibitor (tested sample) wells were charged with 90 μL of lipoxygenase enzyme and 10 μL of respective stock (tested sample) solution. The reaction was initiated by

adding 10 μL of the substrate (AA) to all wells. The plate was then shaken for 5 min on an orbital shaker. Ultimately, 100 μL of chromogen solution (prepared according to standard protocol) was added to each well to stop the enzyme catalysis. The plate was incubated for 30 min and was read at 500 nm. The percentage inhibitions (I%) of the tested sample were calculated using the following equation:

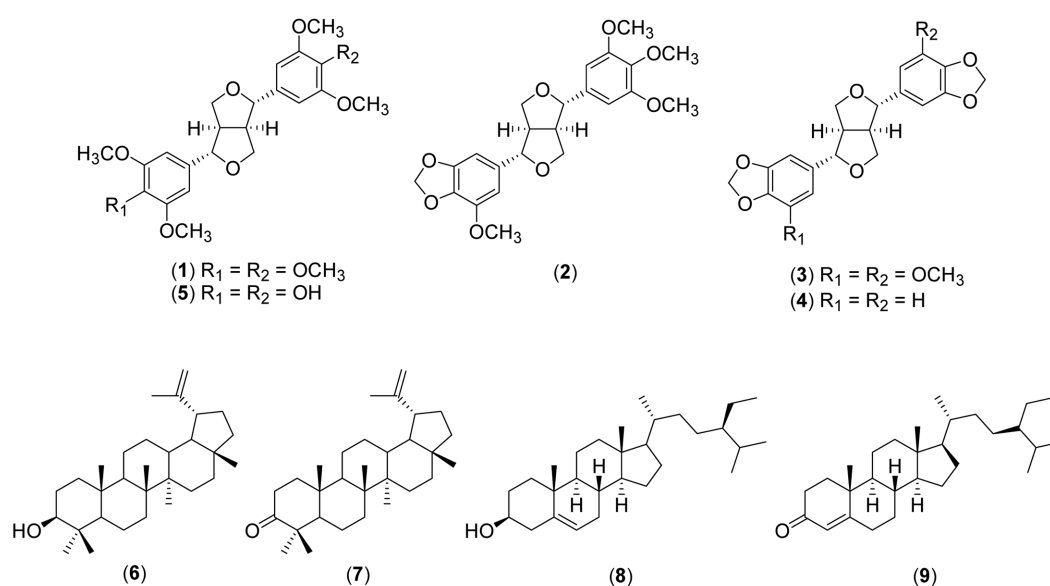
$$I\% = [A_{\text{initial activity}} - A_{\text{inhibitor}} / A_{\text{initial activity}}] \times 100$$

where  $A_{\text{initial activity}}$  is the absorbance of 100% initial activity wells without sample and  $A_{\text{inhibitor}}$  is the absorbance of sample/reference. Analyses were expressed as means ± SD of triplicates.

**Statistical analysis** – Data obtained from biological activity was expressed as mean values. The statistical analyses were carried out by employing one way ANOVA ( $p < 0.05$ ). A statistical package (SPSS version 11.0) was used for the data analysis.

## Result and Discussion

In continuation of our research on the medicinal plants from Malaysian flora, we have performed a phytochemical investigation on the stem bark of a Malaysian Lauraceae, *B. pulverulenta*, which has led to the isolation of nine compounds (Fig. 1), characterised as five lignans two triterpenes, and two steroids. These metabolites were identified by analysing their spectroscopic data and comparing them with the literature data, as (+)-yangambin



**Fig. 1.** Chemical structures of isolated compounds (1 - 9) from *B. pulverulenta*.

**Table 1.** Anticholinesterase and anti-inflammatory activities of lignans from *B. pulverulenta*

Samples/Assays	AChE IC <sub>50</sub> (μM)	BChE IC <sub>50</sub> (μM)	COX-2 IC <sub>50</sub> (μM)	LOX IC <sub>50</sub> (μM)
(+)-Yangambin ( <b>1</b> )	179.8	168.8	33.1	22.4
(+)-Sesartemin ( <b>2</b> )	247.2	240.2	48.7	28.7
(+)-Excelsin ( <b>3</b> )	350.7	256.5	59.4	28.5
(+)-Sesamin ( <b>4</b> )	504.2	325.7	32.7	30.3
(+)-Syringaresinol ( <b>5</b> )	239.7	232.5	27.9	21.0
Galantamine	49.8	57.4	–	–
Indomethacin	–	–	21.3	–
Quercetin	–	–	–	0.5

(**1**),<sup>12</sup> (+)-sesartemin (**2**),<sup>12</sup> (+)-excelsin (**3**),<sup>13</sup> (+)-sesamin (**4**),<sup>14</sup> (+)-syringaresinol (**5**),<sup>14</sup> lupeol (**6**),<sup>15</sup> lupenone (**7**),<sup>16</sup> β-sitosterol (**8**),<sup>17</sup> and β-sitostenone (**9**).<sup>17</sup> These isolated compounds were found for the first time in this plant. There are several reports on lignans neolignans isolated from the *Beilschmiedia* species, such as *B. tsangii*,<sup>9</sup> *B. volckii*,<sup>21</sup> and *B. kunstleri*.<sup>22</sup> They are also reported to occur in other genus from Lauraceae family such as *Endiandra*,<sup>23</sup> *Litsea*,<sup>24</sup> *Lindera*,<sup>25</sup> *Cinnamomum*,<sup>26</sup> and *Ocotea* species.<sup>27</sup>

Acetylcholinesterase plays an important role in the central nervous system. It is one of the fastest known enzymes and catalyzes the cleavage of acetylcholine in the synaptic cleft after depolarization. Inhibitors of AChE, such as galanthamine, are used frequently in the pharmacotherapy of AD.<sup>28</sup> Previous studies showed that AChE/BChE inhibitory activity was not only limited to alkaloids but also other compounds such as flavonoid, lignans, coumarins and essential oils.<sup>29,30</sup> Lignans (**1** - **5**) were tested for AChE/BChE inhibitory activity using Ellman colorimetric method in 96-well microplate. The results are shown in Table 1 representing their IC<sub>50</sub>. Compound (**1**) showed the best activity with IC<sub>50</sub> values of 179.8 and 168.8 μM for AChE and BChE, respectively. Although the inhibition activities for both enzyme were lower than the positive control, galantamine but higher than the other lignans. The present of six methoxyl groups in (**1**) resulted in higher AChE/BChE activity. This is confirmed by the statement made by Roman,<sup>31</sup> that cholinesterase activity is enhanced by the presence of methoxyl substituents.

Inflammatory and pathogenic condition activates the enzymes cyclooxygenase (COX) and 5-lipoxygenase (5-LOX). COX and 5-LOX are the key enzymes in the synthesis of prostanoid and eicosanoids from poly unsaturated fatty acids (PUFAs), which are involved in various inflammatory and allergic disorders. The importance of the dual inhibition of LOX and COX lies in the

effective reduction of chronic inflammatory conditions.<sup>32</sup> Anti-inflammatory activity of the isolated lignans was determined by using two methods, which were cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) assays. The results are shown in Table 1. All tested lignans showed significant activities towards both assays. Compound (**5**) with two hydroxyl and four methoxyl as the substituents, gave the highest activity on COX-2 assay with IC<sub>50</sub> value of 27.9 μM, comparable to the standard indomethacin, IC<sub>50</sub> of 21.3 μM. In LOX assay, compound (**1**) and (**5**) showed the best activity among the other lignans with IC<sub>50</sub> value of 22.4 and 21.0 μM, respectively, but lower than that of quercetin, IC<sub>50</sub> of 0.5 μM. Cyclooxygenase (COX), an enzyme also known as prostaglandin (PG) H synthase converts arachidonic acid to prostaglandins, which play a crucial role as mediators of inflammatory responses such as cytokines and bacterial endotoxin, lipopolysaccharide (LPS). COX-2 produces large amount of PGE<sub>2</sub>, a key inflammatory mediator and associated with inflammation, carcinogenesis, mitogenesis and several neuronal diseases.<sup>33</sup> On the other hand, lipoxygenase is involved in arachidonic acid metabolism, generating various biologically active leukotrienes that play an important role in inflammation. Inhibition of leukotrienes synthesis on lipoxygenase pathway may contribute in anti-inflammatory activity.<sup>34</sup>

The current finding showed (+)-yangambin (**1**) exerted significant anti-inflammatory and anticholinesterase activities, compared with the remaining lignans. Thus, support the statement by Tibet<sup>4</sup> that cholinesterase inhibition has anti-inflammatory roles. In addition, a more comprehensive understanding should be made to reveal the mode action of lignans which might be helpful in understanding the possible roles in human physiology.

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