New Azafluorenone Derivative and Antibacterial Activities of *Alphonsea cylindrica* Barks

Munirah Abdul Talip, Saripah Salbiah Syed Abdul Azziz, Chee Fah Wong, Khalijah Awang, Humera Naz, Yuhanis Mhd Bakri, Mohamad Syahrizal Ahmad, and Marc Litaudon

1Department of Chemistry, Faculty of Science and Mathematics, Sultan Idris Education University, 35900 Tanjung Malim, Perak Darul Ridzuan, Malaysia
2Department of Biology, Faculty of Science and Mathematics, Sultan Idris Education University, 35900 Tanjung Malim, Perak Darul Ridzuan, Malaysia
3Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
4Atta-ur-Rahman Institute of Natural Product Discovery and Faculty of Pharmacy, University Technology MARA, Puncak Alam, 42300 Selangor, Malaysia
5Centre de Recherche de Gif, Institute de Chimie des Substances Naturelles, CNRS, 1, Avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France

**Abstract** – A phytochemical study of *Alphonsea cylindrica* King (unreported) has led to the isolation of six alkaloids. The compounds were identified as kinabaline (1; azafluorenone alkaloid), muniranine (2), O-methylmoschatoline (3; oxoaporphine alkaloid), lysicamine (4), atherospermidine (5) and N-methylouregidione (6; 4, 5-dioxoaporphine alkaloid). The structures of the isolated compounds were determined based on the spectroscopic techniques and by comparison with data reported in the literature. Alkaloid 2 was isolated as a new derivative of azafluorenone while alkaloids 1, 3 - 6 were isolated for the first time from *Alphonsea* species. In addition, alkaloid 3 and 4 showed inhibition zone against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus cereus* in disc diffusion test. The minimum inhibition concentration (MIC) values of lysicamine (4) against *S. aureus*, *B. cereus* and *P. aeruginosa* were found to be smaller than O-methylmoschatoline (3). Therefore, the reported antibacterial activity showed the potential of this plant as natural antibacterial agent and supported the documented traditional use of *Alphonsea* sp. in the treatment of diarrhea and fever.

**Keywords** – *Alphonsea*, Alkaloids, Antibacterial, Disc diffusion test, MIC

**Introduction**

The genus *Alphonsea* belongs to the family Annonaceae, consisting of small trees and shrubs, found from northeastern India and southern China southwards to Ceylon and Malaysia. There are about 30 species of *Alphonsea*. In Malaysia, the species that can be found are *A. borneensis*, *A. curtisii*, *A. elliptica*, *A. maingayi*, *A. rugosa*, *A. kingii* and *A. cylindrica*. In addition, *A. hainanensis*, *A. monogyna* and *A. tsangyuanensis* have been listed as endangered species. The timber of *Alphonsea* was used in boat-building in India and the fruits are edible. Traditionally, the fruits of *Alphonsea* species were used as febrifuge, emmenagogue and antidysenteric. Previous phytochemical studies had reported the isolation of steroids, terpenoids, isoquinoline and azafluorenone alkaloids from *Alphonsea* species. Moreover, these species were also reported as antioxidant, anticancer, antifungal, anti-inflammatory and antitrypanosomal. *Alphonsea cylindrica* is one of the species which can be found in lowland forest such as Malaysia and locally known as “mempisang” which can grow up to 20 m tall and 0.18 m in diameter. The outer bark is greyish or brownish when mature while the inner bark is pale yellow or brown in color. The leaves are 6.5 - 11.5 cm × 3 - 5 cm in size, elliptic and alternate simple (Fig. 1A). It has shining dark green color on the upside leaves while pale green on the downside. The fruits are globose to cylindrical and hairy (Fig. 1B). However, investigation on chemical constituents including alkaloids of this plant has yet to be established and thus requiring more research.

*Author for correspondence*
Saripah Salbiah Syed Abdul Azziz, Department of Chemistry, Faculty of Science and Mathematics, Sultan Idris Education University, 35900 Tanjung Malim, Perak Darul Ridzuan, Malaysia
Tel: +6015-48117313; E-mail: saripahl@fsmt.upsi.edu.my

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This paper deals with the isolation of alkaloid compounds from the barks of *A. cylindrica* and their antibacterial activity. This is the first report describing the phytochemical investigations of *A. cylindrica* and its bioactivity. Six alkaloids were successfully isolated and identified as *kinabaline* (1), *muniranine* (2), *O*-methylmoschatoline (3), *lysicamine* (4), *atherospermidine* (5), and *N*-methylouregidione (6) (Fig. 2). Muniranine (2) was isolated as a new derivative of azafluorenone while alkaloids 1, 3-6 were isolated for the first time from *Alphonsea* species. Based on the spectroscopic analysis and comparison with the reported data in literature, alkaloids 1-2 were identified as azafluorenones while alkaloids 3-5 were oxoaporphines and alkaloid 6 was a 4, 5-dioxoaporphine.

**Experimental**

**General experimental procedures** – The mass spectra were recorded using Gas chromatography/Mass Spectrometry Agilent Technologies GCMS-5975C VL MSD spectrometer (Santa Clara, California, USA) and Liquid Chromatography-Tandem Mass Ion Trap Agilent Technology 1200 Series (Santa Clara, California, USA). The IR spectra were recorded on a Thermo Scientific FTIR model 6700 spectrophotometer using ATR diamond. The $^1$H, $^{13}$C, and 2D (COSY, H MBC, HMQC) Nuclear Magnetic Resonance (NMR) spectrum were carried out using JEOL ECX (500 MHz). Aluminum supported silica gel 60 F254 plates were used for thin-layer chromatography (TLC), visualized under ultraviolet light (254 and 365 nm) and sprayed using Dragendorff’s reagent. For column chromatography, silica gel 60, 200 - 400 mesh, was used while preparative thin layer chromatography use silica gel 60 containing gypsum.

**Plant materials** – *Alphonsea cylindrica* with herbarium number KL 5379 was collected from Kechau Tui, Kuala Lipis, Pahang in March 2007. Species was identified and collected by Phytochemical group of Chemistry Department, University of Malaya, Kuala Lumpur. The voucher specimen was deposited at the Chemistry Department, University of Malaya, Kuala Lumpur.

**Extraction and isolation** – The dried and powdered bark (2000 g) were defatted with hexane at room temperature, to give hexane extract (7.20 g) after removal of the solvent. After that, the bark residue was wetted with 30% ammonia solution and left for two hours. Then, the bark was re-extracted with CH$_2$Cl$_2$, followed by MeOH, to give CH$_2$Cl$_2$ (11.21 g) and MeOH (47.68 g) extracts after solvent removal by using a rotary evaporator. A portion of CH$_2$Cl$_2$ (7.91 g) extract was further acidified by the addition of 5% HCl solution (3 L) and basified with 10% ammonium solution until pH 11 was reached and extracted with CH$_2$Cl$_2$ until a negative Mayer test obtained. From the acid base extraction, alkaloid and neutral fraction was obtained. The brown reddish gum of alkaloid extract (1.2 g) was chromatographed on silica gel chromatography (CC), eluted with increasing concentration of MeOH in CH$_2$Cl$_2$ to yield 335 fractions (20 mL each). These fractions were evaluated and pooled according to TLC analysis yielding 43 fractions (A1 - A43). Fraction A14 was subjected to preparative TLC eluted with CHCl$_3$: EtOAc (90:10, v/v) with ammonia vapours, affording 1 (5.7 mg). Fraction A16 was rechromatographed on silica gel CC and eluted with CH$_2$Cl$_2$, CH$_2$Cl$_2$/EtOAc mixtures, EtOAc, EtOAc/MeOH mixtures and MeOH to obtain 2 (5.1 mg). Purification of fraction A18 by silica gel CC, eluted with a gradient system consisting of increasing concentrations of EtOAc in CHCl$_3$ (100:0 to 10:90, v/v) followed by MeOH in EtOAc (100:0 to 10:90, v/v) yielded 3 (2.3 mg) while purification of fraction A21 by CC using the same condition as above gave 4 (3.3 mg). Fraction A24 yielded 5 (1.7 mg) after being purified on

![Fig. 1. (a) Herbarium of A. cylindrica; (b) Fruits of A. cylindrica.](image-url)

![Fig. 2. Structures of alkaloids 1 - 6.](image-url)
silica gel CC with increasing concentrations of EtOAc in CHCl₃ (100:0 to 10:90, v/v) followed by MeOH in EtOAc (100:0 to 10:90, v/v). Neutral fraction of dichloromethane extract (4.0 g) that obtained from the acid base extraction was also rechromatographed on silica gel by increasing the gradient of mobile phase; hexane, hexane/dichloromethane mixtures, dichloromethane, dichloromethane/methanol mixtures and methanol to give 11 fractions. Then, fraction 9 was further purified by CC with normal silica gel as stationary phase and gave alkaloid 6 (1.1 mg).

**Kinabahine (1)** – Yellow amorphous; ¹H, ¹³C NMR: See Table 1; Positive mode EI-MS m/z 271.1 [M⁺]; (cited for C₁₅H₁₉NO₄, 271.268).

**Muniranine (2)** – Yellow amorphous; ¹H, ¹³C NMR: See Table 1; Negative mode HR-ESI-MS m/z 300.0869 [M⁺]; (cited for C₉H₁₉NO₄, 301.294).

**O-methylmoschatoline (3)** – Orange amorphous; ¹H NMR (CDCl₃ with 1% v/v TMS, 500 MHz) δ (ppm): 9.11 (1H, d, J = 7.5 Hz, H-11), 8.89 (1H, d, J = 5.5 Hz, H-5) 8.58 (1H, d, J = 7.5 Hz, H-8), 7.79 (1H, d, J = 5.0 Hz, H-4), 7.76 (1H, t, H-10), 7.58 (1H, t, H-9), 7.21 (1H, s, H-3), 4.09 (3H, s, OCH₃), 4.01 (3H, s, OCH₂). ¹³C-NMR (CDCl₃ with 1% v/v TMS, 125 MHz) δ (ppm): 182.7 (C-7), 156.6 (C-1), 148.5 (C-3), 147.4 (C-2), 145.5 (C-6a), 144.5 (C-5), 134.6 (C-11a), 134.5 (C-10), 131.5 (C-7a), 131.2 (C-3b), 129.0 (C-8), 128.2 (C-9), 127.7 (C-11), 122.9 (C-3a), 119.3 (C-4), 115.7 (C-1a), 61.9 (OCH₃-3), 61.6 (OCH₂-2), 61.1 (OCH₂-1). Positive EI-MS m/z 321.1 [M⁺]; (cited for C₁₉H₂₃NO₄, 321.327)

**Lysicamine (4)** – Yellow amorphous powder; ¹H-NMR (CDCl₃ with 1% v/v TMS, 500 MHz) δ (ppm): 9.17(1H, d, J = 7.5 Hz, H-11), 8.89 (1H, d, J = 5.5 Hz, H-5) 8.58 (1H, d, J = 7.5 Hz, H-8), 7.79 (1H, d, J = 5.0 Hz, H-4), 7.76 (1H, t, H-10), 7.58 (1H, t, H-9), 7.21 (1H, s, H-3), 4.09 (3H, s, OCH₂-1), 4.01 (3H, s, OCH₂-2).

**Table 1. NMR spectroscopic data for alkaloids 1 and 2 in CDCl₃ (500 MHz ¹H and 125 MHz ¹³C)**

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<th>HMBC</th>
<th>COSY</th>
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<th>δ¹H</th>
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<td>(br s, 1H)</td>
<td>C-6, C-4b, C-7</td>
<td>C7</td>
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were sealed with parafilm and inverted before incubation at 37°C for 17.3 h. The tubes were then incubated for 17.3 h and read with spectrophotometer (Thermo Scientific BIOMATE 3S UV-Visible Spectrophotometer). The MIC of the selected compounds was determined by measuring the optical density at 620 nm, the amount of bacteria growth in each tube is compared to that of the uninoculated or negative growth control.

**Minimum inhibition concentration (MIC)**

The broth dilution method was employed to determine the minimum inhibition concentration (MIC) of the isolated compounds that exhibit antibacterial activity from the disc diffusion assay. This method was previously described by EUCAST. A two-fold serial dilution for the compounds was completed using disc diffusion assay as previously described by Sati (2021).

Compounds 3 - 6 structures were elucidated by comparing their 1H, 13C NMR, and MS spectral data with those in the literatures and determined as O-methylmoschatoline (3),27 lysicamine (4),28 atherospermidine (5)29 and N-methylouregidione (6).30 Alkaloid 2 was isolated as a new derivative of azafluorenone while alkaloid 1, 3 - 6 were isolated for the first time from Alphonsea species.

Kinabaline (1) was isolated as yellow amorphous powder. The EIMS analysis exhibited a molecular ion peak at m/z 271.1 [M]+, suggesting the molecular formula C12H11NO4. The IR spectrum gives strong absorption bands for hydroxyl and carbonyl group at 3308 and 1660 cm⁻¹ respectively. The 1H NMR spectrum (CDCl3, 500 MHz) of 1 displayed resonance of methyl singlet (CH3-1; δ 2.61), two singlets of methoxyl (OCH3-2; δ 3.91, OCH3-8; δ 3.99), one singlet of aromatic proton of ring C (H-7; δ 6.32), two doublets of aromatic protons of ring A (H-2; δ 6.94, J = 5.0 Hz and H-3; δ 8.50, J = 5.0 Hz) and one broad singlet of hydroxyl group (OH-6; δ 8.81). These 1H NMR chemical shifts were found to be in full agreement with those reported for kinabaline (6-hydroxy-5, 8-dimethoxyxynchene).31 The 13C NMR spectrum of 1 exhibited fifteen signals. Signal at δ 194.0 attributed to carbonyl group while signals at δ 62.0 and 56.7 indicated two carbons of methoxyl groups and a signal at δ 17.3 showed the presence of methyl group. The 13C NMR chemical shifts were also compared with darienine (7-hydroxy-5, 8-dimethoxyxynchene) which indicated that C-6 displays a higher field resonance (δ 101.5) when compared to that (δ 155.8) in alkaloid 1. The hydroxyl group at location of C-6 in 1 affects the resonance to be more downfield. The analysis of 1D and 2D NMR spectra led to the assignment of carbon and hydrogen signals as tabulated in Table 1.

Muniranine (2) gave an ion peak at m/z 300.0869 [M-H]+ in the LCMS spectrum indicating the molecular formula of C10H12NO3. The IR spectrum showed strong absorption at 3305 and 1676 cm⁻¹ due to the presence of hydroxyl and carbonyl groups, respectively. The 1H NMR spectrum of 2, revealed a singlet of methyl (δ 2.59; CH3-1) and three singlets of methoxyl (δ 4.04; OCH3-8), (δ 4.01; OCH3-9) and (δ 3.98; OCH3-7). In addition, alkaloid 2 has two doublets of aromatic protons positioned at δ 6.89 (J = 5.0 Hz, H-2), δ 8.45 (J = 5.0 Hz, H-3) and one broad singlet of hydroxyl group at δ 8.71 (OH-6). The HMBC spectrum of 2 showed correlation between methoxyl of OCH3-5 (δ 61.8) with C-5 (δ 154.7). In the same analysis, methoxyl OCH3-7 (δ 61.5) also revealed correlation with
the carbons C-7 and C-8. Moreover, correlation between OH-6 with carbons at δ 143.8 (C-7), 113.8 (C-4b), 144.0 (C-8), 148.8 (C-6) and 154.7 (C-5) were also observed in the HMBC spectrum. Therefore, this observation allowed us to determine the correct position of methoxyls and hydroxyl group of 2 (Fig. 3). In addition, the NOESY correlations were observed between hydrogen of OH-6 to hydrogen of OCH$_3$-7, hydrogen of CH$_3$-1 with H-2 and H-3 while COSY spectrum exhibited cross peak between H-2 and H-3. The analysis of 1D and 2D NMR spectra of alkaloid 2 led to the full assignment of all carbons and protons (Table 1). Generally, the pattern of NMR spectra of alkaloids 1 and 2 are similar. Methyl signal for both compounds was assigned at C-1 in ring A because the small $J$ value for H-2 and H-3, 5.0 Hz showed that these protons were adjacent to the electronegative atom, nitrogen with ortho position. Besides that, the methoxyl group at C-5 is more deshielded than the methoxyl group at C-7 due to the proximity of pyridine ring. Therefore, alkaloid 2 is identified as a derivative of alkaloid 1 and appeared as a new derivative of azafluorenone alkaloid named muniranine or 6-hydroxy-5,7,8-trimethoxyonychine.

Alkaloids 1 - 6 were tested for antibacterial activity by disc diffusion assay at concentration 1 mg/mL. Only alkaloids 3 and 4 showed antibacterial activity against three microorganisms, *S. aureus, B. cereus* and *P. aeruginosa* (Table 2). Both alkaloids 3 and 4 exhibited the largest inhibition zone against *P. aeruginosa* with diameter zone of inhibition 9.67 ± 0.58 mm and 13.33 ± 1.53 respectively. After that, alkaloids 3 and 4 were further tested to determine the minimum inhibition concentration (MIC) against *S. aureus, B. cereus* and *P. aeruginosa* by broth dilution technique and the optical density of the microorganism was analyzed by spectrophotometer. MIC values were summarized in Table 3. This results was in agreement with study by Yusof et al., Tan et al., and Omar et al., who reported that O-methylmoschatoline and lycsiname exhibited antibacterial activity while atherospermidine was inactive. Moreover, the MIC value of 4 against *S. aureus, B. cereus* and *P. aeruginosa* was observed to be smaller than 3. Previous study by Tavares et al. reported that different substitution pattern in the alkaloid compounds of same basic skeleton may influence their antimicrobial activity. From Fig. 2, it was noticed that oxoaporphine alkaloids, 3 - 5 have the same basic skeleton but different substitution patterns. Compound 3, with methoxyl group at carbon C-1, C-2 and C-3 of ring A and compound 4 with methoxyl group at carbons C-1 and C-2 were found to be active against three microorganisms tested. However, compound 5, which features the methylenedioxy group at position C-1, C-2 and methoxyl group at C-3 were inactive against all the microorganisms tested. Therefore, these results suggested that the substitution at carbons C-1, C-2 and C-3 of ring A in oxoaporphine alkaloid could be important for antibacterial activity. In addition, this is the first antibacterial activity test on compounds 1, 2 and 6. However, these compounds were found inactive against all the microorganisms tested.

**Table 2. Diameter zone of inhibition for alkaloids 1 - 6 against *S. aureus, B. cereus, S.typhi, E. coli* and *P. aeruginosa***

<table>
<thead>
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<th>Alkaloids</th>
<th><em>S. aureus</em></th>
<th><em>B. cereus</em></th>
<th><em>S.typhi</em></th>
<th><em>E. coli</em></th>
<th><em>P. aeruginosa</em></th>
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<tr>
<td>3</td>
<td>8.33 ± 0.58</td>
<td>9.33 ± 0.58</td>
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<td>9.67 ± 0.58</td>
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<tr>
<td>4</td>
<td>11.33 ± 0.58</td>
<td>10.00 ± 1.00</td>
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**Table 3. MIC of alkaloids 3 and 4 against *S. aureus, B. cereus* and *P. aeruginosa***

<table>
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<tr>
<th>Alkaloids</th>
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References

(6) IUCN Red List of Threatened Species. 2010.

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