Two New isoquinoline alkaloids from the bark of *Alphonsea cylindrica* King and their antioxidant activity

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Abstract

Two new isoquinoline alkaloids, iraqine (1) and kareemine (2), along with five known alkaloids, muniranine (3), kinabaline (4), O-methylmoschatoline (5), atherospermidine (6) and N-methylouregidione (7), were purified from the dichloromethane crude extract of *Alphonsea cylindrica* bark. The structures of these compounds were elucidated through extensive 1D and 2D NMR, IR and LC-MS studies and comparison with previously reported data. Compounds (1-5 and 7) were tested for their antioxidant activity using in vitro DPPH radical scavenging assay, and 1, 3 and 4 showed the highest antioxidant activities with IC50 values of 48.77 ± 1.01, 44.51 ± 1.12 and 64.28 ± 0.93 μg/ml, respectively.

1. Introduction

*Alphonsea* is a small genus belonging to the Annonaceae family. According to The Plant List (2013), approximately 37 *Alphonsea* species have been discovered thus far, and these species are mostly scattered in the Indo–Malayan regions and China (Talip et al., 2017; Aldulaimi et al., 2018). *Alphonsea cylindrica* King, locally known as ‘mempisang’, is mainly found in lowland forests and not widely dispersed. Previous studies have reported that *Alphonsea* species present various properties, such as antifungal (Dsd et al., 2018), antioxidant, anticancer (Dsd et al., 2018; Dodapaneni et al., 2018), anti-inflammatory (Attiq et al., 2017) and antibacterial (Talip et al., 2017) activities. Moreover, the fruits of *Alphonsea* species are traditionally used as an emmanogogue and in antidiarrheal and antipyretic treatments (Bakri et al., 2017). The species is rich in alkaloids, such as aporphine and oxoaporphine (Tadic et al., 1987). Isoquinoline alkaloids are one of the largest groups of plant alkaloids that derived from the structure that signifies the major type of alkaloids which can be commonly discovered in Annonaceae. They have numbers of crucial pharmaceutical agents such as berbamine (Sankaranarayanan et al., 2018), tetrandrine (Shi et al., 2018), coptisine (Bing et al., 2017). According to Nasrullah et al. (2013) isoquinoline alkaloids have been reported to possess effective in scavenging radicals (antioxidant properties). In 2017, we reported a new azafluorenone alkaloid from *A. cylindrica*, muniranine (3) (Talip et al., 2017). We believe that different types of interesting alkaloids have yet to be extracted from this species. Therefore, the present work aims to discover new active compounds from the plant. In this paper, two rare oxoaporphine and bisbenzylisoquinoline alkaloids are reported.

2. Results and discussion

The DCM crude extract of the bark of *A. cylindrica* was purified through open column chromatography (CC) over silica gel to produce two new alkaloids together with five known alkaloids. The known alkaloids were identified by spectroscopic techniques and comparison with previously reported data; these alkaloids include muniranine (3) (Talip et al., 2017), kinabaline (4) (Tadic et al., 1987), O-methylmoschatoline (5) (Kimia, 2015; Taha et al., 2018; Albarracin et al., 2017), atherospermidine (6) (Chen et al., 2018; Kaur and Kaur, 2017; Orozco-Castillo et al., 2016; Zhou et al., 2015) and N-methylouregidione (7) (Sesang et al., 2014; Aziz et al., 2016).

Compound 1 was isolated as a yellow amorphous solid, and its alkaloid property was proven by the positive result observed after spraying with Dragendorff’s reagent. The LC–MS spectrum of 1 exhibited a molecular ion peak at m/z 337.2076 [M−H]+, which conforms to a molecular formula of C18H15NO5. The IR spectrum of the...
The compound showed strong absorption bands at 3420, 2917, 1661 and 1225 cm⁻¹, corresponding to OH, C=H, CO and C=O groups, respectively. The ¹H-NMR spectrum (Table 1) of 1 displayed five aromatic protons. Two triplets appeared at δ 7.59 and 7.79, corresponding to the protons H-9 and H-10, respectively. Another two signals observed as doublets at δ 8.61 and 9.15 (1H, d, J = 8.0) were allocated to H-8 and H-11, respectively. The methoxy group at C-1 caused a deshielding effect, which led to the downshifted signal of H-11. A singlet (1H) at δ 8.75 was referred to H-5. Very often, oxoaporphine H-4 and H-5 appear as a pair of doublets with a coupling constant of 5 Hz. However, in the structure of compound 1, H-4 was replaced with a methoxy group. The ¹³C-NMR spectrum (Table 1) of compound 1 showed 3 methoxyl carbons, 5 methine carbons, 10 quaternary carbons and 1 carbonyl carbon. The three methoxyl carbons appeared at δ 60.63, 61.16 and 61.26 and were attributed to OCH₃-1, OCH₃-3 and OCH₃-4, respectively. The five methine carbon atoms, C-11, C-10, C-9, C-8 and C-4, were observed at δ 128.05, 134.68, 128.55, 128.99 and 144.22, respectively. Ten quaternary carbon signals at δ 116.26, 130.15, 122.42, 131.34, 134.21, 144.94, 134.82, 149.18, 150.19 and 156.62 were assigned to C-1a, C-3a, C-3b, C-7a, C-11a, C-6a, C-2, C-3, C-4 and C-1, respectively. A carbonyl carbon at C-7 was identified at δ 182.61 (Fig. 1).

The compound was further confirmed by 2D NMR analyses (COSY, NOESY, HMQC and HMBC) (Fig. 2). The NOESY spectrum of the compound showed correlations between OCH₃-4 and OCH₃-3, thereby confirming the location of OCH₃ at C-4; another correlation between OCH₃-1 with H-11 supported the supposition that the methoxy group was located at C-1. The HMBC spectrum of 1 displayed correlations between H-5 and C-3a and C-6a. The structure of compound 1 was very similar to that of 4-hydroxy-1,2,3-trimethoxy-7H-dibenzo-quinolin-7-one, which was previously isolated from the aerial parts of Houttuynia cordata (Ma et al., 2017), except that the position of the OH group in compound 1 was located at C-2. Compound 1 was 2-hydroxy-1,3,4-trimethoxy-7H-dibenzo-quinolin-7-one named as iraqiine.
Protons for two monomers **A** and **B** were obtained. Monomer **A** was an aporphine, as evidenced by two multiplet signals appearing at 3.09 and 3.17, which were ascribed to H-4 and H-5, respectively. A pair of triplets at 7.28 and 7.47, corresponding to two protons each, were identified as H-9 and H-10 respectively. Two other doublet signals at δ 7.12 and 9.62 (J = 8.5 and 8.0 Hz) were assigned to H-8 and H-11, respectively. The high chemical shift of H-11 could be due to hydrogen bonding with the oxygen atom of the methoxyl group at C-1. Three single signals of methoxy groups were observed at δ 4.02, 3.86 and 2.97, thereby suggesting OCH3-1, OCH3-2 and OCH3-3, respectively.

Monomer **B** appeared to be an oxoaporphine. The appearance of a singlet signal at δ 8.83 was attributed to H-5′, and two doublets of aromatic protons appeared at δ 8.62 (H-8′, J = 8.0 Hz) and 9.15 (H-11′, J = 8.0 Hz). In addition, two triplet signals at δ 7.77 and 7.58 were attributed to H-10′ and H-9′, respectively. Three singlet signals at δ 4.01, 4.12 and 4.08 confirmed the existence of three methoxy groups at C-1′, C-2′ and C-3′, respectively.

The 13C-NMR spectra revealed 39 carbon signals corresponding to 6 methoxyl carbons at δ 60.53, 61.10, 61.17, 61.43, 61.23 and 60.94 belonging to OCH3-1, OCH3-1′, OCH3-2, OCH3-2′, OCH3-3 and OCH3-3′, respectively; 9 methine carbons C-11, C-11′, C-10, C-10′, C-9, C-9′, C-8, C-8′ and C-5′ at δ 127.21, 127.88, 124.96, 134.44, 126.12, 128.19, 125.30, 128.78 and 149.17, respectively; 21 quaternary carbons at δ 151.06, 156.45, 122.38, 115.88, 146.21, 148.30, 149.20, 150.98, 151.06, 156.45, 122.38, 115.88, 146.21, 148.30, 149.20, 150.98.
Fig. 3. Antioxidant Activity of isolated compounds.

The dried and powdered bark of A. cylindrica (2.0 kg) was extracted by cool extraction using hexane, DCM and methanol to obtain three different crude extracts according to the polarity of the compounds. The extracts were concentrated using a rotary evaporator to give 10, 25 and 50 g of hexane, DCM and methanol crude extracts, respectively. The DCM extract (8.5 g) was subjected to silica gel CC and eluted with DCM/MeOH (100:1–1:100, v/v), yielding 83 fractions (fractions 1–83). Then, fractions 9–10 were subjected to CC on silica gel using DCM/MeOH (100:0–95:5, v/v) to afford 46 subfractions; subfraction 4 was identified as isoursuline (3.5 mg). Fractions 11–12 were subjected to CC on silica gel using EA/hexane (25:75–50:50, v/v) to afford 180 subfractions. Subfractions 14–48 were subjected to CC on silica gel using DCM/EA (100:0–50:50, v/v) to afford 41 fractions. Subfractions 9–14 were further subjected to CC on silica gel using EA/DCM (5:95, v/v) to give 8 fractions, and fractions 4–6 were labelled kinabalin (10 mg). Subfractions 49–81 from the second column of fractions 11–12 were subjected to CC on silica gel using DCM/MeOH (100:0–95:5, v/v) to afford 25 subfractions. Fractions 5–11 were further subjected to CC on silica gel using EA/DCM (10:90, v/v) to give 25 fractions. Fractions 13–18 were combined and labelled as compound 2 (4.1 mg), whilst fractions 22–25 were labelled muniranine (15 mg). Subfractions 82–101 from the second column of fractions 11–12 were subjected to CC on silica gel using DCM/EA (90:10, v/v) to afford 20 fractions; subfractions 7–20 were then subjected to CC on silica gel using DCM/EA (93:7, v/v) to afford 21 fractions. Subfractions 5–9 were subjected to CC on silica gel using DCM/MeOH (99:1, v/v) to give 8 fractions. Then, subfractions 4–8 were subjected to CC on silica gel using DCM/MeOH (99:5:0.5 v/v) to give 9 fractions; subfractions 2–8 were labelled N-methylouregidinone (3.1 mg). Fractions 13–15 from the first column were subjected to CC on silica gel using DCM/MeOH (100:0 to 95:5, v/v) to afford 95 subfractions. Subfractions 11–19 were subjected to CC on silica gel using DCM/MeOH (99:1, v/v) to afford 7 fractions, and subfractions 1–4 were identified as atherospermidine (2.1 mg). Fractions 19–21 from the first column were subjected to CC on silica gel using DCM/MeOH (100:0–90:10, v/v) to obtain 28 subfractions. Subfractions 11–23 were pored over silica gel CC using DCM/MeOH (98.5:1.5, v/v) mobile phase to produce 14 fractions, and fractions 10–14 were assigned as compound 1 (3.9 mg).

3.4. Antioxidant (DPPH) assay

DPPH assay was conducted according to the method reported by Sahreen et al. (2010) with some modifications. The stock solution, 24 mg of DPPH was dissolving in 100 ml of methanol in a dark room and then stored at 20°C. Different concentrations of isolated compounds (20, 40, 80, 160 or 320 μg/ml) were prepared using methanol. ESI source operating a Sunfire analytical C18 column (150 × 2.1 mm², i.d. 3.5 μm, Waters) was recorded at the University of Paris–Sud France. Dragendorff’s reagent was used to check alkaloid properties. Silica gel 60 (200–400 mesh) was used for CC. Methanol (Merck, Germany), DPPH and ascorbic acid (Sigma–Aldrich Chemical Co., USA) were used to detect DPPH radical scavenging activity.

3.2. Plant material

The bark of A. cylindrica King was collected from Hutan Simpan, Bukit Kinta, Chemor, Perak in March 2007. The species was identified by its phytochemical group at the Chemistry Department, University of Malaya, Kuala Lumpur. A voucher specimen of A. cylindrica (KL 5379) was deposited at the Chemistry Department, University of Malaya, Kuala Lumpur.

3.3. Extraction and isolation

A. K. Obaid Aldulaimi et al.
Then, 2 ml of the prepared solution was placed in a dry test tube, and 3 ml of DPPH solution was added to it with shaking. The mixture was incubated at room temperature for 30 min in the dark to complete the reaction. The absorbance of the mixture was measured at 517 nm using a spectrophotometer against a blank. In the positive control, the sample was substituted with ascorbic acid. All tests were carried out in triplicates, and the results were expressed in μg/ml. DPPH scavenging activity was calculated using the following equation.

Scavenging activity (%) = \( \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100 \)

where:
- \( A_{\text{blank}} \) is the absorbance of blank
- \( A_{\text{sample}} \) is the absorbance of samples

\( IC_{50} \) was estimated from the graph plotted against the percentage inhibition and compared with the standard.

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**Appendix A. Supplementary data**

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2018.11.022.

**References**


