CHEMICAL CONSTITUENTS OF THE LEAVES OF ACTINODAPHNE PRUINOSA

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ABSTRACT. This study was designed to investigate the chemical constituents from Actinodaphne pruinosa growing in Malaysia. A phytochemical investigation of the leaves part resulted in the isolation of boldine (1), norboldine (2), laurotetanine (3), reticuline (4), syringaresinol (5), lupeol (6), and taraxerol (7). The structures of the isolated phytochemicals were established by analysis of their spectroscopic data, as well as the comparison with that of reported data. Notably, this is the first time to report the isolation and structural elucidation of the constituents from the leaves part of A. pruinosa.

KEY WORDS: Actinodaphne pruinosa, Aporphine, Lauraceae, Phytochemical

INTRODUCTION

The genus Actinodaphne belongs to the family Lauraceae and involves 70 species of evergreen trees and shrubs. It occurs mainly in tropical-subtropical Asia and is widely distributed in Malaysia, Indonesia, Eastern Asia, and a few are found in North America [1]. It is locally known as wuru (Indonesia) or medang kuning and medang kunyit (Malaysia) [2]. This genus has been reported to produce isoquinoline alkaloids [3], lactones [4], lignans [5], and phenolic amides [6]. We have recently reported the essential oil compositions and biological activities of this species [7]. The GC and GC-MS analysis of A. pruinosa leaf essential oil resulted in the identification of 28 chemical components, representing 71.6% of the total oil. The major components were globulol (17.8%) and spathulenol (12.0%). The essential oil of A. pruinosa demonstrated significant activity on DPPH (IC50 85.6 μg/mL), phenolic content (190.2 mg GA/g), and lipoxygenase (IC50 85.2 μM) assays. In addition, antioxidant, antityrosinase, acetylcholinesterase, and anti-inflammatory activities of the leaves and stem bark extracts have also been reported [8]. The methanolic leaves extract of A. pruinosa have shown activity on DPPH (IC50 176.8 μg/mL), ABTS (IC50 224.5 μg/mL), and phenolic content (42.8 mg gallic acid/g). Besides, the extracts have also shown significant inhibitory activity against mushroom tyrosinase (I: 44.6%), lipoxygenase (I: 40.8%), and acetylcholinesterase (I: 75.8%). Previously, Rachmatiah et al. [9]

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have been successfully reported the isolation and characterization of new aporphine; (+)-N-(2-hydroxypropyl)-lindcarpine, together with boldine, norboldine, lindcarpine, and methylindcarpine which were obtained from dichloromethane extract of the stem bark of *A. pruinosa*. The new compound also exhibited significant cytotoxicity against P-388 murine leukemia cells. In continuation of our search for bioactive compounds from Malaysian flora [10-14], we have investigated a phytochemical study on the leaves part of *A. pruinosa*.

## RESULTS AND DISCUSSION

Chemical constituents on the leaves part of *A. pruinosa* species which has led to the isolation of seven compounds, which are characterized as four aporphine alkaloids, one lignan, and two steroids. These metabolites were identified by analyzing their spectroscopic data and comparing them with the literature data. Four aporphine alkaloids elucidated as boldine (1), norboldine (2), laurotetanine (3), reticuline (4), a lignan syringaresinol (5), and two triterpenes of lupeol (6) and taraxerol (7) (Figure 1).

Figure 1. Chemical structures of isolated compounds from *A. pruinosa*.

Compound 1 was obtained as amorphous powder (5.0 mg) and showed a molecular ion peak at *m/z* 328 for a formula of C<sub>19</sub>H<sub>21</sub>NO<sub>4</sub>. The UV and NMR spectroscopic data of 1 and 2 were almost identical. Close inspection between the ¹H and ¹³C NMR spectra of 2 and 1 revealed that the only difference between these two alkaloids was due to the additional signal at δ<sub>H</sub> 2.55 and δ<sub>C</sub> 43.2 in the spectra of 1. This led to the assumption that the hydrogen which was initially bonded to N-6 in 2 was replaced by a methyl group in 1. Extensive analysis of all spectroscopic data established enabled the complete assignment of all the ¹H and ¹³C signals of alkaloid, which eventually led to the identification of the compound as boldine (1).

Compound 2 was obtained as brownish amorphous solid (4.5 mg). The EI-MS spectrum showed a molecular ion peak at *m/z* 314 corresponding to the molecular formula of C<sub>18</sub>H<sub>19</sub>NO<sub>4</sub>. The ¹H and ¹³C NMR spectra showed considerable similarities with that of laurotetamine (3) indicating that the two structures are closely related to one another. However, for compound 2, C-2 attach to hydroxyl group instead of a methoxyl group. The actual distribution of OH and OCH<sub>3</sub>
substituents were determined by using HMBC spectrum. The correlation of H-3 to C-1 and C-2, H-11 to C-9 and H-8 to C-10 thus proving the oxygenation pattern for the ring A was 1-methoxyl-2-hydroxyl and for ring D was 9-hydroxyl-10-methoxyl. Compound 2 was, therefore, assigned as norbodine (2).

Compound 3 was obtained as a brownish amorphous powder (6.2 mg). The EIMS spectrum displayed a molecular ion peak at m/z 328, compatible with the molecular formula of C_{16}H_{20}NO_4. The 1H NMR spectrum was identical to that of N-methylauratetine, except for the lack of the N-CH_3 signal that resonated at δ_6 2.72 [9]. In addition, the signal of H-6a that appeared at δ_6 3.82 shifted downfield indicating the presence of an NH group adjacent to the methine H-6a proton. In addition, the absence of the signal at δC 44.0 belong to N-CH_3 in the 13C-NMR spectrum of N-methylauratetine [9] and the apparent of IR absorption at v_{max} 3429 cm^{-1} thus established the presence of secondary amine group as 6-NH. Compound 3 was, therefore, assigned as lauratetine (3).

Compound 4 was obtained as a brownish amorphous powder (5.5 mg). The UV spectrum exhibited an absorption maximum at λ_{max} 285 nm which suggested the presence of a benzyloquinoline moiety. The EIMS showed a molecular ion peak at m/z 330 corresponding to a molecular formula of C_{16}H_{20}NO_4. The 1H-NMR spectrum displayed signals corresponding to five aromatic protons, together with two OCH_3, one N-CH_3, one CH_2-CH_2-N, one shielded methine proton, and one isolated methylene group. The signals in the aromatic region were ascribed to the singlets of H-5 (δ_6 5.51) and H-8 (δ_6 3.67) of ring A, and the three protons of ring C with a AMX spin system forming a dd centered at H-6’ (δ_6 5.57) ortho-coupled to H-5’ (δ_6 6.71) and meta-coupled to H-2’ (δ_6 1.75). The two methoxyl signals were present at δ_6 3.83 that corresponding to six protons. The former was attached to C-6, while the latter to C-4’. A singlet, appeared at δ_6 2.42, attributed to N-CH_3. Subsequently, H-1 resonated as a dd at δ_6 3.63-3.66, while, H_α-α resonated as a dd at δ_6 2.69-3.02 and H_β-α as a doublet at δ_6 2.74. The 13C NMR spectrum showed 19 carbon signals comprising six methine, seven quaternary, three methylene, two methoxyl, one N-CH_3 carbon. Compound 4 was, therefore, assigned as reticuline (4).

Compound 5 was isolated as colorless needles (15.9 mg). The 1H NMR and COSY spectra were similar to the 1H NMR and COSY spectra of known compound, yangambin except the methoxyl groups at C-4 and C-4’ were replaced by two hydroxyl groups. The present of this hydroxyl groups was represented by a singlet at δ 5.56 (2H, s). The 13C NMR spectrum displayed the presence of eight signals corresponding to twenty two carbons in the molecule. The DEPT spectra assigned these signal to eight quaternary, eight methines, two methylenes and four methoxyl carbons. The HMBC spectrum revealed the correlations between H-8 (δ 3.11) with C-8, C-7’, C-8’ and C-1, while H-7’ (δ 4.75) correlate with C-8’, C-9’, C-1’, C-2’ and C-6’. The 13C NMR results were supported by the EIMS spectrum which gave molecular ion peak at m/z 418, consistent with a molecular formula C_{22}H_{26}O_{8}. Compound 5 was, therefore, assigned as syringaresinol (5).

Alkaloids broadly exist in nature and have a typical pharmacological activity. Many of these isolated aporphine alkaloids were isolated previously from the genus Actinodaphne. Compound (1) and (2) have been isolated from *A. pruinosa* [9], compound (3) from *A. obovata* [3], compound (4) from *A. macrophylla* [15], whilst compound (5) was previously isolated from *A. lancifolia* [16]. The isolated terpenoids were also reported most of the *Actinodaphne* species. They were readily identified by comparison of physical and spectroscopic data and mass spectrometry data with values found in the literature [15-17].

**EXPERIMENTAL**

*Plant material.* *A. pruinosa* leaves were collected from Hutan Simpan Bangi, Selangor in September 2015, and identified by Dr. Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (SK2957/16) was deposited at the Herbarium of IBS, UPM.

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**General procedures.** Solvents systems used in the chromatographic method were n-hexane, chloroform (CHCl₃), dichloromethane (DCM), and methanol (MeOH). Soxhlet extraction technique was applied to extract the phytochemicals from the dried sample. Vacuum liquid chromatography (VLC) was performed on Merck silica gel 60 (230-400 mesh) while column chromatography (CC) on Merck silica gel 60 (70-230 mesh) was the stationary phase. Thin-layer chromatography (TLC) analysis was performed on Merck precoated silica (SiO₂) gel F₂₅₄ plates with 0.2 mm thickness to detect and monitor compounds present in the samples. The spots were visualized under UV light at 254 and 365 nm, and spraying reagent vanillin-sulfuric acid in MeOH followed by heating. The ¹H (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 Spectrophotometer. Chemical shifts were reported in ppm and CDCl₃ as the solvent. Residual solvent was used as an internal standard. The IR spectra were recorded on Perkin Elmer ATR and 1600 spectrophotometer series as KBr disc or thin film of NaCl discs. Mass spectral data were obtained from the Mass Spectrometry Service, National University of Singapore (NUS).

**Extraction and isolation.** The dried and powdered leaves part of *A. pruinosa* (400 g) was extracted consecutively by a soxhlet extractor with hexane (5 L), DCM (5 L), and MeOH (5 L). Evaporation of the respective solvents gave hexane (1.5 g, 0.38%), DCM (3.5 g, 0.88%), and MeOH (5.2 g, 1.30%) extracts. The hexane extract (1.5 g) was subjected to vacuum liquid chromatography (VLC) on SiO₂ 60 (230-400 mesh) using hexane and CHCl₃ in 5% increasing polarity to give 5 fractions (APH1-5). The combined fractions of APH2-3 were purified by column chromatography (CC) on silica gel 70-230 mesh to afford compounds (6) (12 mg) and (7) (15 mg). The crude DCM was fractionated by VLC on SiO₂ 70-230 mesh, using hexane and CHCl₃ in 10% increasing polarity to give 8 fractions (APD1-8). The combined fractions APD4-6 were purified and recrystallized in hexane:CHCl₃ to yield compounds (1) (5 mg) and (2) (5 mg). Besides, the combined fractions of APD7-5 afforded compound (5) (7 mg). The crude MeOH was fractionated by VLC on SiO₂ 70-230 mesh, using CHCl₃:MeOH in 10% increasing polarity to give five fractions (APM3-5). The combined fractions APM3-4 were purified by CC to yield (3) (4 mg) and (4) (5 mg).

**Boldine (1).** Light brown powder. MS m/z: 328 [M⁺, C₁₃H₁₇NO₄], m.p. 162-164 °C [9]; IR νmax cm⁻¹: 3430 (N-H), 1510, 1465 (C=C), 1235 (CO); UV (MeOH) λmax nm: 282, 305; ¹H NMR (400 MHz, CDCl₃, δ, ppm, J/Hz): δ 2.55 (3H, s, N-CH₃), 2.40-2.75 (3H, m, H-7b, H-4), 2.92-3.10 (4H, m, H-6a, H-5, H-7a), 3.60 (3H, s, 2-OCH₃), 3.90 (3H, s, 10-OCH₃), 6.65 (1H, d, H-3), 6.80 (1H, d, H-8), 7.92 (1H, s, H-11); ¹³C NMR (100 MHz, CDCl₃, δ, ppm): δ 28.5 (C-4), 35.0 (C-7), 43.2 (N-CH₃), 53.0 (C-5), 56.0 (1-OCH₃), 62.5 (C-6a), 110.0 (C-11), 113.0 (C-3), 114.0 (C-8), 123.5 (C-11c), 126.0 (C-11b), 126.5 (C-11a), 130.0 (C-3a), 130.0 (C-7a), 142.0 (C-1), 143.0 (C-9), 145.2 (C-10), 148.0 (C-2).

**Norboldine (2).** Brownish amorphous solid. MS m/z: 314 [M⁺, C₁₃H₁₇NO₄], m.p. 138-140 °C [9]; IR νmax cm⁻¹: 3420 (NH), 1505, 1462 (C=C), 1236 (CO); UV (MeOH) λmax nm: 282, 305; ¹H NMR (400 MHz, CDCl₃, δ, ppm, J/Hz): δ 2.77-3.80 (6H, m, H-4a/4b,5a/5b,7a/7b), 3.60 (3H, s, 1-OCH₃), 3.85 (3H, s, 10-OCH₃), 4.12 (1H, dd, J = 14.0, 4.4, H-6a), 6.65 (1H, s, H-3), 6.75 (1H, s, H-8), 8.02 (1H, s, H-11); ¹³C NMR (100 MHz, CDCl₃, δ, ppm): δ 29.2 (C-4), 36.5 (C-7), 43.0 (C-5), 53.8 (C-6a), 56.0 (10-OCH₃), 60.2 (C1-OCH₃), 110.2 (C-11), 113.5 (C-3), 114.0 (C-8), 123.5 (C-11a), 125.0 (C-1a), 128.0 (C-1b), 130.0 (C-7a), 130.5 (C-3a), 141.8 (C-1), 145.0 (C-9), 145.5 (C-10), 148.2 (C-2).

**Laurotetanin (3).** Brownish amorphous solid. MS m/z: 328 [M⁺, C₁₃H₁₇NO₄], m.p. 125-127 °C; IR νmax cm⁻¹: 3429 (NH), 1508, 1464 (C=C), 1238 (CO) [3]; UV (MeOH) λmax nm: 215, 285, 305; ¹H NMR (400 MHz, CDCl₃, δ, ppm, J/Hz): δ 2.71 (2H, m, H-7), 3.02 (2H, m, H-4), 3.28 (1H, s br, NH), 3.38 (2H, m, H-5), 3.65 (3H, m, 1-OCH₃), 3.82 (1H, m, H-6a), 3.88 (3H, m, 2-OCH₃), 3.92 (3H, s, 10-OCH₃), 5.67 (1H, s, H-8).

from this species may be used as chemotaxonomic markers for this species. Meanwhile, the high variants of alkaloids compounds from this species. The phytochemical investigation from the leaves part of A. pruinosa furnished four aporphine alkaloids, together with a lignan and two steroids. This study is the first report of the occurrence of a lignan from this species. Actinodaphne species. The plants of the Lauraceae tend to produce alkaloids, lactones, and lignan, but generally not these compounds. This study is the first report of the occurrence of a lignan from this species. A. pruinosa furnished four aporphine alkaloids, together with a lignan and two steroids. This study is the first report of the occurrence of a lignan from this species. Actinodaphne species. The plants of the Lauraceae tend to produce alkaloids, lactones, and lignan, but generally not these compounds.
groups of compounds in a single plant. However, there are also examples of plants of this family (such as *A. lancifolia*) where lactones and lignans coexist. The next step will be to evaluate the biological activities of the isolated compound/extracts in order to valorize this species with a special ecological character. In addition, to validate the biological activity, clinical trials should be carried out to ensure the safe use of the compounds as therapeutic agents. This study also provides valuable and useful information and indications for further exploring the potential nutraceutical and pharmaceutical applications of the genus Lauraceae.

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