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SHORT COMMUNICATION

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 (IC_{50} 85.6 μg/mL), phenolic content (190.2 mg GA/g), and lipoxygenase (IC_{50} 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 (IC_{50} 176.8 μg/mL), ABTS (IC_{50} 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 methyllindcarpine 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).

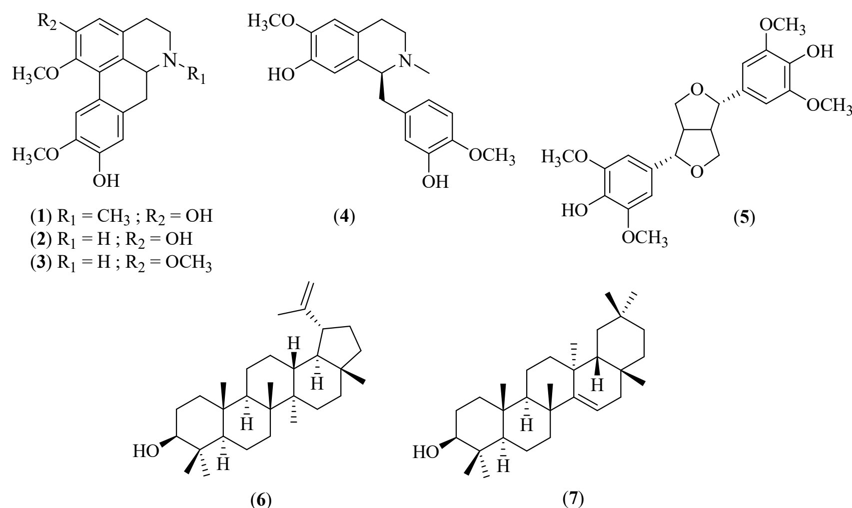

Figure 1. Chemical structures of isolated compounds from *A. pruinosa*.

Compound **1** was obtained as amorphous powder (5.0 mg) and showed a molecular ion peak at *m/z* 328 for a formula of $C_{19}H_{21}NO_4$. The UV and NMR spectroscopic data of **1** and **2** were almost identical. Close inspection between the ^{1}H and ^{13}C NMR spectra of **2** and **1** revealed that the only difference between these two alkaloids was due to the additional signal at δ_H 2.55 and δ_C 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 ^{1}H and ^{13}C 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 EIMS spectrum showed a molecular ion peak at *m/z* 314 corresponding to the molecular formula of $C_{18}H_{19}NO_4$. The ^{1}H and ^{13}C NMR spectra showed considerable similarities with that of laurotetanine (**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_3

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 norboldine (**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 $C_{19}H_{21}NO_4$. The ^{1}H NMR spectrum was identical to that of *N*-methyllaurotetanine, except for the lack of the *N*-CH_3 signal that resonated at δ_H 2.72 [9]. In addition, the signal of H-6a that appeared at δ_H 3.82 shifted downfield indicating the presence of an NH group adjacent to the methine H-6a proton. In addition, the absences of the signal at δ_C 44.0 belong to *N*-CH_3 in the ^{13}C-NMR spectrum of *N*-methyllaurotetanine [9] and the apparent of IR absorption at ν_{max} 3429 cm^{-1} thus established the presence of secondary amine group as 6-NH. Compound **3** was, therefore, assigned as laurotetanine (**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 benzylisoquinoline moiety. The EIMS showed a molecular ion peak at *m/z* 330 corresponding to a molecular formula of $C_{19}H_{23}NO_4$. The ^{1}H-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_H 6.51) and H-8 (δ_H 6.37) of ring A, and the three protons of ring C with a AMX spin system forming a *dd* centered at H-6′ (δ_H 6.57) *ortho*-coupled to H-5′ (δ_H 6.71) and meta-coupled to H-2′ (δ_H 6.75). The two methoxy signals were present at δ_H 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_H 2.42, attributed to *N*-CH_3. Subsequently, H-1 resonated as a *dd* at δ_H 3.63-3.66, while, H_A-α resonated as a *dd* at δ_H 2.69-3.02 and H_B-α as a doublet at δ_H 2.74. The ^{13}C 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 colurless needles (15.9 mg). The ^{1}H NMR and COSY spectra were similar to the ^{1}H 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 ^{13}C 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 ^{13}C 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.

General procedures. Solvents systems used in the chromatographic method were *n*-hexane, chloroform ($CHCl_3$), 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 (SiO_2) gel F_{254} 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 ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 Spectrophotometer. Chemical shifts were reported in ppm and $CDCl_3$ 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 SiO_2 60 (230-400 mesh) using hexane and $CHCl_3$ 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 SiO_2 70-230 mesh, using hexane and $CHCl_3$ in 10% increasing polarity to give 8 fractions (APD1-8). The combined fractions APD4-6 were purified and recrystallized in hexane:$CHCl_3$ to yield compounds (**1**) (5 mg) and (**2**) (5 mg). Besides, the combined fractions of APD7-8 afforded compound (**5**) (7 mg). The crude MeOH was fractionated by VLC on SiO_2 70-230 mesh, using $CHCl_3$:MeOH in 10% increasing polarity to give five fractions (APM1-5). 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^+, $C_{19}H_{21}NO_4$]. m.p. 162-164 °C [9]; IR υ_{max} cm^{-1}: 3430 (N-H), 1510, 1465 (C=C), 1235 (CO); UV (MeOH) λ_{max} nm: 282, 305; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): δ 2.55 (3H, s, *N*-CH_3), 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-OCH_3), 3.90 (3H, s, 10-OCH_3), 6.65 (1H, s, H-3), 6.80 (1 H, s, H-8), 7.92 (1H, s, H-11); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): δ 28.5 (C-4), 35.0 (C-7), 43.2 (*N*-CH_3), 53.0 (C-5), 56.0 (10- OCH_3), 60.5 (1-OCH_3), 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), 145.0 (C-9), 145.2 (C-10), 148.0 (C-2).

Norboldine (2). Brownish amorphous solid. MS *m/z*: 314 [M^+, $C_{18}H_{19}NO_4$]. m.p. 138-140 °C [9]; IR υ_{max} cm^{-1}: 3420 (NH), 1505, 1462 (C=C), 1236 (CO); UV (MeOH) λ_{max} nm: 282, 305; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): δ 2.77-3.80 (6H, m, H-4a/4b/5a/5b/7a/7b), 3.60 (3H, s, 1-OCH_3), 3.85 (3H, s, 10-OCH_3), 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); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): δ 29.2 (C-4), 36.5 (C-7), 43.0 (C-5), 53.8 (C-6a), 56.0 (10-OCH_3), 60.2 (C1-OCH_3), 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).

Laurotetanine (3). Brownish amorphous solid. MS *m/z*: 328 [M^+, $C_{19}H_{21}NO_4$]. m.p. 125-127 °C; IR υ_{max} cm^{-1}: 3429 (NH), 1508, 1464 (C=C), 1238 (CO) [3]; UV (MeOH) λ_{max} nm: 215, 285, 305; ^{1}H NMR (400 MHz, $CDCl_3$, 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-OCH_3), 3.82 (1H, m, H-6a), 3.88 (3H, m, 2-OCH_3),

3.87 (3H, m, 10-OCH_3), 6.59 (1H, s, H-3), 6.82 (1H, s, H-8), 8.05 (1H, s, H-11); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): δ 29.0 (C-4), 36.5 (C-7), 43.0 (C-5), 53.5 (C-6a), 55.8 (10-OCH_3), 56.0 (2-OCH_3), 60.2 (1-OCH_3), 110.5 (C-3), 111.0 (C-11), 113.8 (C-8), 124.0 (C-11a), 126.5 (C-1a), 127.5 (C-1b), 128.5 (C-3a), 129.2 (C-9), 129.5 (C-7a), 144.2 (C-1), 145.5 (C-10), 152.0 (C-2).

Reticuline (4). Brownish amorphous solid. MS *m/z* 330 [M^+, $C_{19}H_{23}NO_4$]. m.p. 130-131 °C; IR υ_{max} cm^{-1}: 3053 (NH), 2931 (CH), 1663 (CC), 1036 (CO) [15]; UV (MeOH) λ_{max} nm: 285, 305; ^1H NMR (400 MHz, $CDCl_3$): δ 2.42 (3H, s, *N*-CH_3), 2.53-2.82 (2H, m, H-4), 2.69-3.02 (1H, dd, *J* = 14.0, H_A-α), 2.74 (1H, d, *J* = 14.0, H_B-α), 2.69-3.18 (2H, m, H-3), 3.63-3.66 (1H, dd, *J* = 14.0, 6.0, H-1), 3.83 (6H, s, 4'/6-OCH_3), 6.37 (1H, s, H-8), 6.51 (1H, m, H-5), 6.57 (1H, dd, *J* = 2.0, 8.0, H-6'), 6.71 (1H, d, *J* = 8.0, H-5'), 6.75 (1H, d, *J* = 2.0, H-2'); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.0 (C-4), 41.0 (C-α), 42.5 (*N*-CH_3), 46.7 (C-3), 55.9 (4'/6-OCH_3), 64.5 (C-1), 110.4 (C-5), 110.6 (C-5'), 113.7 (C-8), 115.6 (C-2'), 120.9 (C-6'), 125.3 (C-8a), 130.3 (C-4a), 133.2 (C-1'), 143.3 (C-7), 145.1 (C-6), 145.2 (C-3'), 145.3 (C-4').

Syringaresinol (5). Colurless needles. MS *m/z* 418 [M^+, $C_{22}H_{26}O_8$]. m.p. 183-184 °C [16]; IR υ_{max} cm^{-1}: 3340 (OH), 2923 (CH), 1567, 1462 (C=C), 1262 (CO); UV (MeOH) λ_{max} nm: 259, 281; ^1H NMR (400 MHz, $CDCl_3$): δ 3.11 (2H, ddd, *J* = 9.2, 6.8, 4.4, H8/H-8′), 3.91 (12H, s, 3/3′/5/5′-OCH_3), 3.93 (2H, unresolved, H-9b/H-9′b), 4.30 (2H, dd, *J* = 9.2, 6.8, H-9a/H-9′a), 4.75 (2H, d, *J* = 4.4, H-7/H-7′), 5.56 (2H, s, 4/4′-OH), 6.60 (4H, s, H-2/H-2′/H-6/H-6′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 54.3 (C-8/C-8′), 56.3 (3/3′/5/5′-OCH_3), 71.8 (C-9/C-9′), 86.0 (C-7/C-7′), 102.7 (C-2/C-6/C-2′/C-6′), 132.1 (C-1/C-1′), 134.3 (C-4/C-4′), 147.1 (C-3/C-5/C-3′/C-5′).

Lupeol (6). White needles. GC-MS *m/z* 426 [M^+, $C_{30}H_{50}O$]. m.p. 215-216 °C [17]; IR υ_{max} cm^{-1}: 3434 (OH), 2927 (CH), 1634 (C=C), 1070 (CO); ^1H NMR (400 MHz, $CDCl_3$): δ 0.71 (1H, d, *J* = 9.2, H-5), 0.77 (3H, s, H-28); 0.80 (3H, s, H-25), 0.94 (3H, s, H-27), 0.96 (3H, s, H-23), 0.98 (3H, s, H-24), 1.00 (3H, s, H-26), 1.67 (3H, s, H-30), 1.95 (2H, m, H-21), 2.36 (1H, dt, *J* = 11.2, 5.6, H-19), 3.19 (1H, dd, *J* = 11.2, 5.4, H-3), 4.58 (1H, s, H-29), 4.70 (1H, s, H-29); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.5 (C-27), 15.3 (C-24), 15.9 (C-26), 16.1 (C-25), 18.0 (C-28), 18.3 (C-6), 19.3 (C-30), 20.9 (C-11), 25.1 (C-12), 27.3 (C-23), 27.4 (C-2), 27.9 (C-15), 29.8 (C-21), 34.3 (C-7), 35.5 (C-16), 37.1 (C-10), 38.0 (C-13), 38.7 (C-1), 38.8 (C-4), 40.0 (C-22), 40.8 (C-8), 42.8 (C-14), 43.0 (C-17), 47.9 (C-19), 48.3 (C-18), 50.4 (C-9), 55.3 (C-5), 79.0 (C-3), 109.3 (C-29), 150.9 (C-20).

Taraxerol (7). White solid. GC-MS *m/z* 426 [M^+, $C_{30}H_{50}O$]. m.p. 282-283 °C [17]; IR υ_{max} cm^{-1}: 3053 (CH), 2931 (CH), 1663 (C=C), 1036 (CO); ^1H NMR (400 MHz, $CDCl_3$): δ 0.82 (3H, s, H-25); 0.84 (3H, s, H-28), 0.88−2.03 (23H, m, overlapping CH and CH_2), 0.92 (3H, s, H-26), 0.94 (3H, s, H-30), 0.97 (3H, s, H-24), 0.99 (3H, s, H-29), 1.11 (6H, s, H-27/H-23), 3.20 (1H, dd, *J* = 11.2, 4.8, H-3), 5.54 (1H, dd, *J* = 8.0, 3.2, H-15); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.4 (C-24). 15.4 (C-25), 17.4 (C-11), 18.8 (C-6), 21.3 (C-30), 25.8 (C-26), 27.1 (C-2), 27.9 (C-23), 28.7 (C-20), 29.8 (C-27), 29.9 (C-28), 33.1 (C-22), 33.3 (C-29), 33.7 (C-7), 35.1 (C-16), 35.7 (C-13), 36.6 (C-21), 37.5 (C-10), 37.7 (C-1), 37.9 (C-12), 38.0 (C-4), 38.7 (C-17), 38.9 (C-8), 41.3 (C-19), 48.7 (C-18), 49.3 (C-9), 55.5 (C-5), 79.0 (C-3), 116.8 (C-15), C 158.1 (C-14).

CONCLUSION

In the present study, 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. Meanwhile, the high variants of alkaloids compounds from this species may be used as chemotaxonomic markers for this *Actinodaphne* species. The plants of the Lauraceae tend to produce alkaloids, lactones, and lignan, but generally not these

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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