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SHORT COMMUNICATION

Chemical Constituents from *Diospyros argentea* Griff. (Ebenaceae)

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Abstract

Diospyros argentea is distributed mainly in Southeast Asia where it has been used to repel mosquitoes and its pounded roots are applied externally to treat herpes zoster. Chemical investigation of *D. Argentea* leaves resulted in the isolation of three steroids, β-sitosterol **(1)**, β-sitostenone **(2)**, and stigmasterol **(3)**, together with five triterpenes, identified as friedelin **(4)**, taraxerol **(5)**, taraxerone **(6)**, betulin **(7)**, and lupeol **(8)**. Their structures were elucuidated using spectroscopic analysis and chemical evidence. To the best of our knowledge, all compounds were isolated for the first time from this species.

Keywords: *Diospyros argentea*, Ebenaceae, phytochemical, steroid, triterpene

INTRODUCTION

Diospyros (Ebenaceae family) is a genus of over 700 species of deciduous and evergreen trees, shrubs, and small bushes. These species are commonly known as ebony or persimmon trees. Some are valued for their hard, heavy, dark timber, and some for their fruits. Some are useful as ornamentals, and many are of local ecological importance (Burkill, 1966). They are also widely-used in traditional African medicine, mainly against leprosy. *Diospyros argentea* is locally known as '*bedil lalat'* in Malaysia. It can be found in lowland and hill forests up to c.a 800 m. and is distributed mainly in Southeast Asia, such as Peninsular Malaysia, Borneo, and Singapore. Leaves of *D. argentea* have been used to repel mosquitoes and their pounded roots are externally applied to treat herpes zoster (Burkill, 1966). Previous phytochemical studies of *Diospyros* species reported

triterpenes (Kuo et al., 1997), naphthoquinones (Cai et al., 2000), anthraquinones (Srivastava and Pitre, 1985), coumarins (Gu et al., 2004), and flavonoids (Mallavadhani and Mahapatra, 2005). Recently, the chemical compositions of the essential oil from this species have been reported (Salleh and Khamis, 2021). GC and GC-MS analysis of *D. argentea* leaf essential oil resulted in the identification of forty components (93.5%) with a high concentration of oxygenated sesquiterpenes (50.9%). The oil was characterised by the abundance of caryophyllene oxide (22.5%), benzyl benzoate (10.7%), α-bisabolene (6.0%), (2*Z*,6*E*)-farnesol (5.5%), and germacrene D (5.2%). As part of our continuing search to explore natural compounds from *Diospyros* species, we have performed the current phytochemical study of *D. argentea* leaves.

MATERIALS AND METHODS

Plant Materials

D. argentea leaves were collected from Gambang, Pahang in September 2019 and identified by Shamsul Khamis, and the voucher specimen (SK38/19) was deposited at the UKMB herbarium.

General Experimental Procedures

A Soxhlet extraction technique was applied to extract phytochemicals from the dried sample. Column chromatography (CC) was performed on Merck silica gel 60 (70-230 mesh) as the stationary phase. Thin-layer chromatography (TLC) analysis was performed on Merck precoated silica gel F_{254} plates with 0.2 mm thickness to detect and monitor compounds in samples. Solvent systems used in the chromatographic method were *n*-hexane, chloroform, dichloromethane, and methanol. The spots were visualised under UV light at 254 and 365 nm, and spraying reagent vanillin-sulphuric acid in MeOH followed by heating. For spectroscopic analysis, ^{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 NMR solvent. Residual solvent was used as an internal standard. IR spectra were recorded on Perkin Elmer ATR and1600 spectrophotometer series as KBr discs or thin films of NaCl discs. Liquid chromatography/mass spectrometry (LC/MS) was used to obtain mass spectral data.

Extraction and Isolation

The dried powdered leaves of *D. argentea* (300 g) were consecutively extracted by a Soxhlet extractor with hexane and dichloromethane. Evaporation of the respective solvents gave hexane (4.5 g) and DCM (5.4 g) extracts. The hexane extract was subjected to CC on SiO_2 60 (230-400 mesh) using hexane and $CHCl_3$ in 5% increasing polarity to give 8 fractions (DAH 1-8). The combined fractions of DAH 2-4 were purified by column chromatography on silica gel 70-230 mesh to afford compounds **1** and **2**. The combined fractions of DAH 5-6 were purified by column chromatography on silica gel 70-230 mesh to afford compounds **3** and **4**. The crude DCM was fractionated by CC on SiO_2 70-230 mesh, using hexane and EtOAc with 10% increasing polarity to give 10 fractions (DAD 1-10). The combined fractions DAD 2-4 were purified and recrystallized from hexane:$CHCl_3$ (8:2) to yield compounds **5** and **6**. The combined fractions DAD 5-7 were purified by CC to yield compounds **7** and **8**.

RESULTS AND DISCUSSION

Investigation of the chemical constituents from the leaves of *D. argentea* has led to the isolation of eight compounds. These metabolites were identified by analysing their spectroscopic data and

comparing it with the literature data (Salleh et al., 2021a; Salleh et al., 2021b; Salleh et al., 2021c; Shakri et al., 2021; Romes et al., 2020).

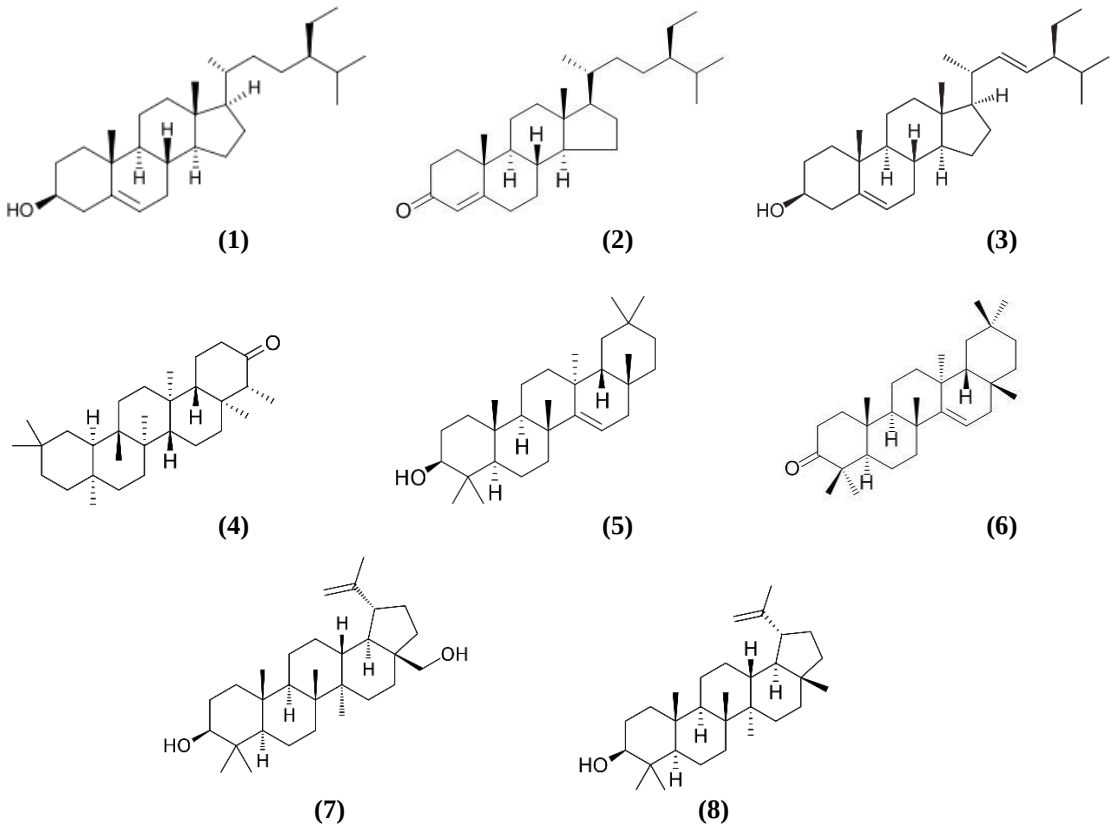

Figure 1. Chemical structures of isolated compounds (**1-8**)

Compound (**1**) was identified as β-sitosterol, white crystalline needles (40.5 mg); m.p 133-134°C; IR (KBr) υ_{max} cm^{-1}: 3435, 2966, 1652, 1052; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.70 (3H, s, H-18), 0.84 (3H, d, J = 6.6 Hz, H-27), 0.86 (3H, d, J = 6.6 Hz, H-26), 0.88 (3H, d, J = 3.9 Hz, H-29), 0.95 (3H, d, J = 6.3 Hz, H-21), 1.03 (3H, s, H-19), 1.27−2.31 (29H, m, overlapping CH and CH_2), 3.54 (1H, m, H-3), 5.37 (1H, d, J = 4.8 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.8 (C-29), 11.9 (C-18), 18.7 (C-21), 19.0 (C-27), 19.3 (C-19), 19.7 (C-26), 21.0 (C-11), 23.1 (C-28), 24.3 (C-15), 26.1 (C-23), 28.2 (C-16), 29.2 (C-25), 31.6 (C-2), 31.9 (C-8), 31.9 (C-7), 33.9 (C-22), 36.1 (C-20), 36.5 (C-10), 37.2 (C-1), 39.8 (C-12), 42.3 (C-4), 42.3 (C-13), 45.8 (C-24), 50.1 (C-9), 56.0 (C-17), 56.7 (C-14), 71.8 (C-3), 121.7 (C-6), 140.7 (C-5); MS *m/z* 414 [M^+, $C_{29}H_{50}O$].

Compound (**2**) was identified as β-sitostenone, white solids (12.8 mg); m.p 77-79°C; IR (KBr) υ_{max} cm^{-1}: 2960, 1687, 1602, 1222; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.73 (3H, s, H-18), 0.82 (3H, d, J = 6.4 Hz, H-27), 0.84 (3H, d, J = 6.4 Hz, H-26), 0.86 (3H, t, J = 7.6 Hz, H-29), 0.93 (3H, d, J = 6.8 Hz, H-21), 1.18 (3H, s, H-19), 1.25−2.44 (29H, m, overlapping CH/CH_2), 5.74 (1H, s, H-4); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.2 (C-29), 14.6 (C-18), 15.9 (C-19), 17.0 (C-21), 18.4 (C-27), 20.8 (C-26), 23.0 (C-11), 24.3 (C-28), 26.2 (C-15), 28.1 (C-23), 29.5 (C-16), 31.9 (C-25), 33.8 (C-7), 35.2 (C-6), 36.2 (C-22), 38.7 (C-2), 39.8 (C-8), 42.5 (C-1), 43.6 (C-20), 44.9 (C-10), 46.0 (C-12), 48.2 (C-13), 49.3 (C-24), 52.0 (C-9), 53.9 (C-17), 56.0 (C-14), 123.7 (C-4), 171.4 (C-5), 198.8 (C-3); MS *m/z* 412 [M^+, $C_{29}H_{48}O$].

Compound (**3**) was identified as stigmasterol, white crystalline needles (28 mg); m.p. 144-145°C; IR (ATR) υ_{max} cm^{-1}: 3379, 2937, 2862, 1055; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95-2.30 (26H, m), 3.54 (1H, m, H-3), 5.03 (1H, dd, J = 15.2 Hz, 8.8 Hz, H-22), 5.17 (1H, dd, J = 15.2 Hz, 8.8 Hz, H-23), 5.37 (1H, br.s, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.0 (C-18), 12.2 (C-29), 19.4 (C-27), 21.1 (C-11), 21.2 (C-19), 23.1 (C-4), 24.3 (C-26), 24.4 (C-15), 25.4 (C-28), 28.9 (C-16), 31.7 (C-2), 31.7 (C-7), 31.9 (C-8), 31.9 (C-25), 36.5 (C-10), 37.3 (C-1), 39.7 (C-12), 40.5 (C-20), 42.4 (C-4), 42.3 (C-13), 50.2 (C-24), 51.2 (C-9), 55.9 (C-17), 56.9 (C-14), 71.8 (C-3), 121.7 (C-6), 129.3 (C-23), 138.3 (C-22), 140.8 (C-5); MS *m/z* 412 [M^+, $C_{29}H_{48}O$].

Compound (**4**) was identified as friedelin, white solid (24.0 mg); m.p. 251-254°C; IR (ATR) υ_{max} cm^{-1}: 2972, 2926, 2868, 1711, 1461, 1389, 1299, 1189, 1073; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.72 (3H, s, H-24), 0.87 (3H, s, H-25), 0.88 (3H, d, J = 6.5 Hz, H-23), 0.95 (3H, s, H-29), 0.99 (3H, s, H-28), 1.00 (3H, s, H-26), 1.05 (3H, s, H-27), 1.18 (3H, s, H-30); ^{13}C NMR (100 MHz, $CDCl_3$): δ 213.2 (C-3), 59.5 (C-10), 58.2 (C-4), 53.1 (C-8), 42.8 (C-18), 42.2 (C-5), 41.5 (C-2), 41.3 (C-6), 39.7 (C-14), 39.3 (C-22), 38.3 (C-13), 37.5 (C-9), 36.0 (C-16), 35.6 (C-11), 35.4 (C-19), 35.0 (C-29), 32.8 (C-21), 32.4 (C-15), 32.1 (C-28), 31.8 (C-30), 30.5 (C-12), 30.0 (C-17), 28.2 (C-20), 22.3 (C-1), 20.3 (C-26), 18.7 (C-27), 18.3 (C-7), 18.0 (C-25), 14.7 (C-24), 6.8 (C-23); MS *m/z* 426 [M^+, $C_{30}H_{50}O$].

Compound (**5**) was identified as taraxerol, white solid (25.5 mg); m.p. 275-277°C; IR (ATR) υ_{max} cm^{-1}: 3484, 3053, 2931, 1663, 1036; ^{1}H 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/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 Hz, H-3), 5.54 (1H, dd, J = 8.0, 3.2 Hz, 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); MS *m/z* 426 [M^+, $C_{30}H_{50}O$].

Compound (**6**) was identified as taraxerone, colourless needles (7.2 mg), m.p. 239-240ºC; IR (KBr) υ_{max} cm^{-1}: 3401, 2936, 2962, 1708, 1457, 1054; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (3H, s, H-30), 0.90 (3H, s, H-24), 0.91 (3H, s, H-29), 0.95 (3H, s, H-28), 1.06 (3H, s, H-23), 1.07 (3H, s, H-26), 1.08 (3H, s, H-25), 1.14 (3H, s, H-27), 5.56 (1H, dd, J = 8.1, 3.3 Hz, H-15); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.8 (C-25), 16.9 (C-11), 20.0 (C-6), 21.2 (C-30), 21.5 (C-24), 25.3 (C-27), 26.1 (C-23), 28.9 (C-20), 29.8 (C-26), 29.9 (C-28), 33.1 (C-22), 33.5 (C-29), 33.7 (C-21), 34.2 (C-2), 35.1 (C-7), 36.1 (C-12), 37.0 (C-16), 37.6 (C-13), 37.9 (C-10), 38.0 (C-17), 38.4 (C-1), 38.9 (C-8), 40.9 (C-19), 47.9 (C-4), 49.0 (C-18), 49.1 (C-9), 55.9 (C-5), 116.3 (C-15), 157.9 (C-14), 218.0 (C-3); MS *m/z* 424 [M^+, $C_{30}H_{48}O$].

Compound (**7**) was identified as betulin, white solid (10.2 mg); m.p. 112-113°C; IR (KBr) υ_{max} cm^{-1}: 3368, 2937, 1646, 1026; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75 (3H, s, H-24), 0.80 (3H, s, H-25), 0.91-1.99 (25H, m, overlapping CH and CH_2); 0.96 (3H, s, H-23), 0.97 (3H, s, H-26), 0.99 (3H, s, H-27), 1.67 (3H, s, H-30), 3.19 (1H, dd, J = 11.2, 5.2 Hz, H-3), 3.34 (1H, d, J = 10.8 Hz, H-28a), 3.81 (1H, d, J = 10.8 Hz, H-28b), 4.60 (1H, d, J = 1.6 Hz, H-29a), 4.70 (1H, d, J = 1.6 Hz, H-29b); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.7 (C-27), 15.3 (C-24), 15.9 (C-26), 16.1 (C-25), 18.3 (C-6), 19.0 (C-30), 20.8 (C-11), 25.2 (C-12), 27.0 (C-15), 27.4 (C-2), 27.9 (C-23), 29.1 (C-16), 29.7 (C-21), 33.9 (C-22), 34.2 (C-7), 37.1 (C-13), 37.3 (C-10), 38.7 (C-4), 38.8 (C-1), 40.9 (C-8), 42.7 (C-14), 47.7 (C-18/17), 48.7 (C-19), 50.4 (C-9), 55.3 (C-5), 60.5 (C-28), 78.9 (C-3), 109.6 (C-29), 150.4 (C-20); MS *m/z* 442 [M^+, $C_{30}H_{50}O_2$].

Compound (**8**) was identified as lupeol, white needles (5.2 mg); m.p. 214-216°C; IR (KBr) υ_{max} cm^{-1}: 3434, 2927, 1634, 1070; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.71 (1H, d, *J* = 9.2 Hz, 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 Hz, H-19), 3.19 (1H, dd, J = 11.2, 5.4 Hz, 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); MS *m/z* 426 [M^+, $C_{30}H_{50}O$].

CONCLUSION

Increasing interest in the chemistry and pharmaceutics of *Diospyros* species may promote new progress in finding and developing novel compounds. The present study is the first phytochemical report for *D. argentea* species including separation and identification of three steroids and five triterpenes from the dried leaves. High variants of triterpenoid compounds from this species may be used as chemotaxonomic markers for this species. Further studies are needed to provide a better understanding of the chemical constituents, mechanism of action and structure activity relationship of *Diospyros* species as a medicinal plant.

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