



CHEMICAL CONSTITUENTS FROM THE LIANAS OF *Gnetum cuspidatum* Blume

(Sebatian Kimia dari Lianas *Gnetum cuspidatum* Blume)

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Abstract

Gnetum is a genus of gymnosperms, the sole genus in the family Gnetaceae with approximately 40 species. Various species has been used for the treatment of rheumatitis, arthritis, bronchitis and asthma in folk medicines. *Gnetum cuspidatum* Blume is known throughout tropical Southeast Asia from Thailand, Vietnam, Cambodia, Malaysia, Sumatra, Java, and Borneo to the Maluku, Sulawesi and New Guinea. In this research work, a methanol extract of the lianas of *Gnetum cuspidatum* was subjected to vacuum liquid chromatography for fractionation. Later, several selective fractions had undergone the repetitive radial chromatography technique for further purification. Four known constituents categorized as stilbene type of compound have been successfully isolated and identified which include resveratrol (1), gnetucleistol C (2), gnetucleistol D (3) and gnetomonol M (4). The structures and configuration of the reported compounds were elucidated on the basis of 2D-NMR correlations and comparison with the literature.

Keywords: Gnetaceae, *Gnetum*, *Gnetum cuspidatum*, stilbene

Abstrak

Gnetum merupakan genus gymnosperms, genus tunggal dalam keluarga Gnetaceae dengan kira-kira 40 spesies. Pelbagai spesies telah digunakan untuk rawatan rheumatitis, arthritis, bronkitis dan asma dalam perubatan penduduk tempatan. *Gnetum cuspidatum* Blume dikenali di seluruh tropika Asia Tenggara dari Thailand, Vietnam, Kemboja, Malaysia, Sumatera, Jawa, Borneo dan ke Maluku, Sulawesi dan New Guinea. Satu ekstrak metanol daripada lianas *Gnetum cuspidatum* telah menggunakan vakum kromatografi cecair untuk pemisahan. Kemudian, beberapa pemisahan yang terpilih telah menjalani teknik kromatografi jejarian secara berulang kali untuk penulenan lanjut. Empat sebatian yang dikategorikan sebagai jenis stilbene telah berjaya diasingkan dan dikenalpasti iaitu resveratrol (1), gnetucleistol C (2), gnetucleistol D (3) dan gnetomonol M (4). Struktur dan konfigurasi sebatian yang telah dilaporkan ini berdasarkan korelasi 2D-NMR dan perbandingan dengan kajian literatur.

Kata kunci: Gnetaceae, *Gnetum*, *Gnetum cuspidatum*, stilben

Introduction

The genus *Gnetum* from the family of Gnetaceae comprises approximately 40 species which were found in the tropical lowlands of the world from Northeastern South America, tropical West Africa, and South China to Southeast Asia. The plants are known to contain stilbenoids or oligostilbene and have been ethnobotanically used as folk medicine as well as food [1]. Previously, many studies have been done on several species of *Gnetum* and

revealed various stilbenes and oligostilbenes type of compounds as its major chemical constituents. Interestingly, some of the isolated compounds display multiple bioactivities such as antioxidant, anti-inflammatory and antitumor [2].

In our search for structurally and biological interesting compounds from tropical plants found in Malaysia, the lianas of *Gnetum cuspidatum* has been investigated. *Gnetum cuspidatum* Blume is known throughout tropical Southeast Asia from Thailand, Vietnam, Cambodia, Malaysia, Sumatra, Java, and Borneo to the Maluku, Sulawesi and New Guinea. Previous study revealed the isolation of isorhapontigenin from the stem [3] and two new compounds recently report by Shimokawa et al. [4] known as cuspidan A and cuspidan B from the bark of this plant.

In this study, the methanol extract of the lianas of this plant has led to the isolation of four known stilbenoid compounds, resveratrol (1) [5], gnetucleistol C (2) [6], gnetucleistol D (3) [7] and gneumonol M (4) [8]. The structures were determined using spectroscopic techniques of 1D and 2D NMR, MS and comparison with previous reports

Materials and Methods

Chemicals and raw materials

All the chemicals used were of analytical grade and purchased from Sigma Chemical Co. (St Louis, Missouri). The lianas of *Gnetum cuspidatum* was collected at Taman Negara, Pahang in August 2011 and identified by Dr Shamsul, a botanist from Universiti Putra Malaysia (UPM), Serdang.

Extraction and isolation of constituents from plant materials

The lianas were air-dried, ground (2 kg) and macerated in methanol which then filtered and concentrated under reduced pressure to obtain crude extract (214.8 g). The crude extract with less tannin (62.60 g) then was subjected to fractionation by using vacuum liquid chromatography (VLC) with various composition of solvent system [n-hexane: EtoAC (7:3, 6:4, 5:5, 3:7, 2:8, 1:9) and EtoAC: MeOH (10:0, 9:1, 8:2)] to yield five major fractions. All pure compounds reported were obtained from one single fraction. Fraction 4 (10.54 g) was subjected to isolation and purification using separation technique of multiple radial chromatography and preparative thin layer chromatography (pTLC) with solvent system of chloroform: methanol (8.5:1.5), and chloroform: acetone (8:2) to yield **1** (1mg), **2** (3.1mg), **3** (6.1mg) and **4** (12.1mg).

Purification and structure elucidation of pure compounds

The structure determination of the constituents isolated from the plants was done using several spectroscopic methods. The ^1H -NMR and ^{13}C -NMR were recorded in acetone- D_6 on Bruker 300 Ultrashield NMR spectrometer measured at 300MHz for ^1H NMR and 75MHz for ^{13}C NMR. Chemical shifts were reported in ppm (δ) and the coupling constants are given in Hz. The mass spectra were measured on Agilent Technologies LC-MS. Melting point was measured by Fisher Johns 'micro melting point apparatus'.

For purification process to obtain a single constituent, various chromatographic techniques have been applied. Vacuum liquid chromatography (VLC) used silica gel 60, 70 – 230 mesh ASTM (Merck 1.07747), column chromatography using Si-gel Merck 60 (200 – 400mesh) and TLC analysis on pre-coated Si-gel plates Si-gel Merck Kiesel gel 60 F254 0.25mm, 20 x 20 cm while glass supported silica gel 60 F₂₅₄ was used for preparative thin layer chromatography.

Results and Discussion

Four known compounds, resveratrol (1), gnetucleistol C (2), gnetucleistol D (3) and gneumonol M (4) (Figure 1) were successfully isolated from the methanol extract of the lianas of *G.cuspidatum*. The known compounds were elucidated on the basis of NMR (^1H and ^{13}C) technique and by comparison of physical and spectroscopic data with values found in the literature. Table 1 displayed all ^{13}C NMR data of the compounds isolated. Resveratrol (1), a naturally occurring stilbene in *Gnetum* plants was obtained as white amorphous powder. ^1H NMR (300 MHz, in Acetone- d_6) revealed two sets of *ortho*-coupled aromatic protons on a *p*-substituted phenyl moieties (ring B) at δ_{H} 7.40 (2H, d, J = 8.7 Hz, H-2, 6) and δ_{H} 6.82 (2H, d, J = 8.4 Hz, H-3, 5). The spectrum also displayed one set of

meta-coupled aromatic proton $\delta_{\text{H}}6.53$ (2H, d, $J = 2.4$ Hz, H-10, 14) and one *meta*-triplet at $\delta_{\text{H}}6.26$ (1H, t, $J = 2.1$, 2.4 Hz, H-12) on a 3, 5-disubstituted benzene ring (ring A). A pair of *trans*-coupled olefinic proton at $\delta_{\text{H}}6.99$ (1H, d, $J = 16.8$ Hz, H-7) / $\delta_{\text{H}}6.90$ (1H, d, $J = 16.2$ Hz, H-8) were also observed.

Gnetucleistol C (2) was obtained as an amorphous white powder. The ^1H NMR spectrum in acetone- d_6 exhibited signals attributable to a methoxy proton at $\delta_{\text{H}}3.95$ (3H, s), an AB_2 system at $\delta_{\text{H}}6.39$ (1H, t, $J = 2.1$ Hz, H-4), $\delta_{\text{H}}6.91$ (2H, d, $J = 2.1$ Hz, H-2, 6), an ABX system at $\delta_{\text{H}}6.75$ (1H, d, $J = 8.1$ Hz, H-11), $\delta_{\text{H}}7.12$ (1H, dd, $J = 8.4$, 0.9 Hz, H-13), $\delta_{\text{H}}7.23$ (1H, t, $J = 8.4$, 8.1 Hz, H-12), and a doublet at $\delta_{\text{H}}7.14$ (1H, d, $J = 0.9$ Hz, H-8), and display a long-range coupling with the signal of C-7 and C-9. Based on comparison with the ^1H and ^{13}C NMR data of gnetifolin A [5], compound 2 was deduced to have a benzofuran skeleton and was categorized under arylbenzofuran monomer type of stilbene.

Gnetuclesitol D (3), also known as 2-methoxyresveratrol was obtained as white amorphous powder. ^1H NMR (300 MHz, in Acetone- d_6) displayed a single peak at $\delta_{\text{H}}3.89$ (3H, s), showing the existence of an additional substituent which was a methoxy group on carbon 2 of ring B. Signals of one set of *meta*-coupled aromatic protons at $\delta_{\text{H}}6.53$ (2H, d, $J = 2.4$ Hz, H-10, 14) and one *meta*-triplet at $\delta_{\text{H}}6.27$ (1H, d, $J = 2.1$ Hz, H-12) were revealed on ring A. The spectrum also showed an ABX system on a 2, 4-disubstituted benzene ring (ring B) at $\delta_{\text{H}}6.79$ (1H, d, $J = 8.1$ Hz, H-6), $\delta_{\text{H}}6.99$ (dd, $J = 2.1$, 8.4 Hz, H-5) and $\delta_{\text{H}}7.21$ (1H, d, $J = 2.1$, H-3). A pair of *trans*-coupled olefinic proton at $\delta_{\text{H}}7.05$ (1H, d, $J = 16.5$ Hz, H-7) / $\delta_{\text{H}}6.89$ (1H, d, $J = 16.2$ Hz, H-8) was also shown.

Gnemonol M (4) was obtained as a colorless amorphous powder. The ^1H NMR spectrum showed the presence of two sets of H-atoms in ABX systems on 1,2,4-trisubstituted benzene rings at $\delta_{\text{H}}6.96$ (1H, d, $J = 1.8$ Hz, H-2a) / $\delta_{\text{H}}6.72$ (1H, d, $J = 8.1$ Hz, H-5a) / $\delta_{\text{H}}6.65$ (1H, dd, $J = 1.8$, 8.1 Hz, H-6a); $\delta_{\text{H}}6.61$ (1H, d, $J = 2.1$ Hz, H-2b) / $\delta_{\text{H}}6.57$ (1H, d, $J = 8.1$ Hz, H-5b) / $\delta_{\text{H}}6.39$ (1H, dd, $J = 2.1$ Hz, 8.1, H-6b). The spectrum also showed two sets of *meta*-coupled H-atoms in AB systems on tetra-substituted benzene rings at $\delta_{\text{H}}6.54$ (1H, d, $J = 2.7$ Hz, H-14a) / $\delta_{\text{H}}6.06$ (1H, d, $J = 1.8$ Hz, H-12a); $\delta_{\text{H}}6.44$ (1H, d, $J = 2.4$ Hz, H-14b) / $\delta_{\text{H}}6.16$ (1H, d, $J = 2.4$ Hz, H-12b). Four benzylic methine singlets at $\delta_{\text{H}}4.20$ (H-7a); 3.69 (H-8a); 4.13 (H-8b); 3.77 (H-7b), two signals for methoxyl groups at $\delta_{\text{H}}3.79$ (OMe-3a); 3.64 (OMe-3b), and phenolic proton at $\delta_{\text{H}}8.02$ was also observed in the spectrum.

Table 1. Spectroscopic data (^{13}C NMR) for compound 1 – 4

No. of carbon	Compounds			
	1	2	3	4
	δ_{C}	δ_{C}	δ_{C}	δ_{C}
1	128.9	133.9	129.5	139.0
2	127.8	105.0	147.8	113.0
3	115.5	160.9	109.3	147.6
4	157.4	105.0	146.7	145.5
5	115.5	160.9	120.3	115.2
6	127.8	105.0	115.1	121.4
7	128.2	159.8	128.5	48.5
8	125.9	100.5	126.2	58.7
9	139.9	121.1	139.9	148.0
10	104.7	155.4	104.7	127.8
11	158.8	105.9	158.7	158.6
12	101.8	127.1	101.9	102.8
13	158.8	105.5	158.7	158.6
14	104.7	157.6	104.7	104.2

Table 1 (cont'd). Spectroscopic data (^{13}C NMR) for compound 1 – 4

No. of carbon	Compounds			
	1	2	3	4
	δ_{C}	δ_{C}	δ_{C}	δ_{C}
OCH_3		56.9	55.4	
OCH_3 (3a)				57.3
OCH_3 (3b)				56.9
1b				136.2
2b				112.3
3b				147.6
4b				145.5
5b				115.2
6b				120.9
7b				52.0
8b				51.2
9b				147.2
10b				113.4
11b				157.9
12b				102.8
13b				157.2
14b				105.7

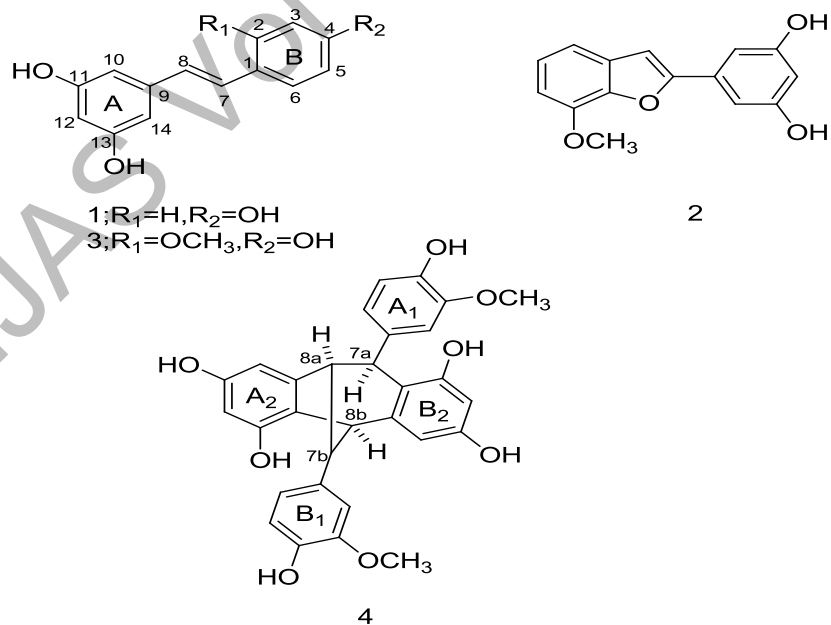


Figure 1. Structure of compound 1 – 4

Conclusion

Four known stilbenoids were successfully isolated and characterized from the methanol extract of the lianas of *Gnetum cuspidatum* Blume. These stilbenoids compounds include resveratrol (1), gnetucleistol C (2), gnetucleistol D (3) and gnemonol M (4). As previous study found that oligostilbenoids from *Gnetum* displayed diverse pharmacological properties, further detailed on its phytochemical investigation is worth to be explored.

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References

1. Medicinal Herb Index in Indonesia. (1995). PT. EISAI Indonesia, Second Edition.
2. Shimizu, K., Kondo, R. and Sakai, K. (2000). Inhibition of tyrosinase by flavonoids, stilbenes and related 4-substituted resorcin. *Planta Medica*, 66: 11–15.
3. Lojanapiwatna, V., Mahaeja, J. and P. Wiriyachitra. (1982). Isolation of (*E*)- 3,4, 5-trihydroxy-3'-methoxystilbene from *Gnetum cuspidatum* Bl. *Journal of the Science Society of Thailand*, 8(1): 59 – 63.
4. Shimokawa, Y., Hirasawa, Y., Kaneda, T., Hamid, A., Hadi, A. and H. Morita. (2012). Cuspidans A and B, Two New Stilbenoids from the Bark of *Gnetum cuspidatum*. *Chemical and Pharmaceutical Bulletin*, 60(6): 790 – 792.
5. Huang, K. S. and Lin, M. (1999). Oligostilbenes from the Roots of *Vitis amurensis*. *Journal of Asian Natural Products Research*, 2: 21 – 28.
6. Yao, C. S., Lin, M., Liu, X. and Wang, Y. H. (2005). Stilbene derivatives from *Gnetum cleistostachyum*. *Journal of Asian Natural Products Research*, 7: 131 – 137.
7. Yao, C. S., Lin, M., Liu, X. and Wang, Y. H. (2003). Stilbene from *Gnetum cleistostachyum*. *Acta Chimica Sinica*, 61 (8): 1331 – 1334.
8. Ilya, I., Ali, Z., Tanaka, T., Iinuma, M., Furusawa, M., Nakaya, K., Murata, J., Darnaedi, D., Matsuura, N. and Ubukata, M. (2003). Stilbene derivatives from *Gnetum gnemon* Linn. *Phytochemistry*, 62: 601 – 606.