separations

MDPI

Article

Separation and Isolation of a New Hydroxylated Resveratrol Trimer Together with Other Stilbenoid Compounds from the Lianas of *Gnetum microcarpum* Blume and Their Inhibitory Effects of Prostaglandin E_2

Nik Fatini Nik Azmin [1], Norizan Ahmat [1,2,*], Juriyati Jalil [3], Carla Wulandari Sabandar [3,4], Nik Khairunissa' Nik Abdullah Zawawi [1], Siti Norafiqah Mohd Sazali [3], Mohd Izwan Mohamad Yusof [1], Aisyah Salihah Kamarozaman [2] and Mulyadi Tanjung [5]

[1] Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Shah Alam 40450, Malaysia; nikfatini88@gmail.com (N.F.N.A.); chemtitude@yahoo.com (N.K.N.A.Z.); izwanyusof@uitm.edu.my (M.I.M.Y.)
[2] Centre of Foundation Studies, Universiti Teknologi MARA Cawangan Selangor, Kampus Dengkil, Dengkil 43800, Malaysia; aisyah2910@uitm.edu.my
[3] Centre for Drug and Herbal Development, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, Kuala Lumpur 50300, Malaysia; juriyatijalil@ukm.edu.my (J.J.); carla@usn.ac.id (C.W.S.); fieqaprissy@gmail.com (S.N.M.S.)
[4] Department of Pharmacy, Faculty of Science and Technology, Universitas Sembilanbelas November Kolaka, Jalan Pemuda, Kolaka 93527, Indonesia
[5] Natural Product Research Group, Organic Chemistry Division, Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya 60115, Indonesia; mulyadi-t@fst.unair.ac.id
* Correspondence: noriz118@uitm.edu.my

Abstract: A new oligostilbene trimer, malaysianol F (**1**), together with ten known stilbenes (**2–11**), were successfully separated and purified from the acetone extract of the lianas of *Gnetum microcarpum*. Malaysianol D (**2**) was isolated for the first time in *Gnetum* plants. The tanninless crude extract (52.5 g) was fractionated using a vacuum liquid chromatography (VLC) technique to give five major fractions. Fraction 2 (4.68 g), 3 (4.79 g) and 4 (9.29 g) were all subjected to further isolation and purification using VLC, column chromatography (CC) and repetitive radial chromatography (RC) techniques with the best solvent system to yield malaysianol F (**1**) (6.2 mg), malaysianol D (**2**) (62.5 mg), malaysianol E (**3**) (2.4 mg), ε-viniferin (**4**) (10 mg), resveratrol (**5**) (6.5 mg), gnetol (**6**) (3.5 mg), gnetucleistol C (**7**) (12.2 mg), isorhapontigenin (**8**) (8 mg), cuspidan B (**9**) (3.2 mg), parvifolol D (**10**) (4.8 mg) and gnetifolin M (**11**) (2.5 mg). Their structures were determined on the basis of the analysis of spectral evidence by extensive NMR data analyses and comparison with the related published data. Several compounds were tested for anti-inflammatory activity. Their inhibitory effect on Prostaglandin E_2 (PGE_2) was tested using radioimmunoassay techniques. Compound **6** exhibited significant concentration-dependent inhibitory effects on PGE_2 production with IC_{50} values of 1.84 μM comparable with the positive control, indomethacin (IC_{50} 1.29 μM).

Keywords: Gnetaceae; *Gnetum microcarpum*; stilbenoids; malaysianol F; prostaglandin E_2

check for updates

Citation: Azmin, N.F.N.; Ahmat, N.; Jalil, J.; Sabandar, C.W.; Zawawi, N.K.N.A.; Sazali, S.N.M.; Yusof, M.I.M.; Kamarozaman, A.S.; Tanjung, M. Separation and Isolation of a New Hydroxylated Resveratrol Trimer Together with Other Stilbenoid Compounds from the Lianas of *Gnetum microcarpum* Blume and Their Inhibitory Effects of Prostaglandin E_2. *Separations* **2023**, *10*, 496. https://doi.org/10.3390/separations10090496

Academic Editor: Marcello Locatelli

Received: 6 August 2023
Revised: 3 September 2023
Accepted: 7 September 2023
Published: 12 September 2023

1. Introduction

Gnetaceae are a family of the most advanced members of tropical gymnosperms in the Gnetales order (Gnetophyta division). They are valued for their taxonomic distinctiveness and outstanding biological interest [1]. They are composed of a sole genus, *Gnetum*, which is represented by about 40 species confined to the tropical and humid regions of the world [2]. Their distribution includes the tropical lowlands of the world, from northeast South America, tropical West Africa and south China to Southeast Asia [3]. Most plants of this family are climbers with twining stems, while the others are shrubs or trees [4]. Numerous species

in this family have been used as folk medicine for the treatment of arthritis, bronchitis and asthma. The leaves and the fruits are also used as food in many parts of the tropics [5]. This plant family is well known as a rich source of plant-derived stilbenoids, which possess unique structures and multi-faceted biological activities, together with Cyperaceae, Dipterocarpaceae, Leguminosae and Vitaceae [6]. Stilbenoid compounds are useful as chemotoxic agents, metabolites and constitutive defense agents and have been found to demonstrate a broad range of biological activities due to their possible pharmacological qualities, including anti-cancer, anti-inflammatory and antioxidant effects [7].

The phytochemical research concerning stilbenoids developed quickly in recent years, leading to the study of the lianas of *G. microcarpum* Blume. The species distribution includes western Indochina (Thailand, Myanmar) and the Malay Peninsula to Sumatra and the nearby archipelagos Lingga Islands, Riau Islands and Anambas Islands [8]. This large-fruited species of climbers grows in Malaysia and has been found in open margins and canopies of secondary forests, on hill slopes and in heath forests. It occurs in altitudes from close to sea level to up to 2000 m, e.g., Gunung Tahan, the highest mountain of the Malay Peninsula [9]. The plant was not recorded in folk medicines. In the present study, we describe in detail the structural elucidation of the new stilbene trimer, malaysianol F (**1**), which was isolated and purified by a combination of vacuum liquid chromatography and repetitive radial chromatography techniques. All of these compounds (**1**–**11**) were isolated for the first time in this plant. Compound **2** was previously reported in *Dryobalanops* plants [10] but never in *Gnetum* plants. In addition, five of the isolated compounds were subjected to radioimmunoassay to determine their PGE_2 inhibitory activity, which is further discussed in the results and discussion section.

2. Materials and Methods

2.1. Plant Material

The lianas of *Gnetum microcarpum* were collected from Tasik Bera, Pahang (3.1298562, 02.6083095), in August 2010. The plant was collected and identified by Dr. Shamsul, a botanist from Universiti Putra Malaysia (UPM), Serdang, and a voucher specimen (SK 2711/01) has been preserved at the Herbarium of the Laboratory of Natural product, Institute of Bioscience, UPM, Serdang, Selangor, Malaysia.

2.2. Chemicals

The chromatographic separation and purification were conducted using the following adsorbents: Silica gel 60 PF_{254} (Merck 1.07747) for vacuum liquid chromatography (VLC), silica gel Merck 60 (0.040–0.063 mm, 230–400 mesh ASTM, Merck 1.09385) for column chromatography, silica gel 60 PF_{254} containing gypsum (1.07749) for preparation of radial chromatography plate and Sephadex LH-20 (lipophilic sephadex, Sigma-Aldrich, Massachusetts, USA) for size exclusion chromatography and TLC analysis on precoated Si-gel plates Si-gel Merck Kieselgel 60 F_{254} 0.25 mm, 20 × 20 cm (1.05554), while glass-supported silica gel 60 F_{254} was used for preparative thin layer chromatography. The spot on the TLC analysis was detected using CeSO4 and H_2SO_4 spray reagents. The solvents used for extraction and isolation were of analytical and industrial grade, purchased from Merck (Darmstadt, Germany), whereby the industrial grade solvents, such as n-hexane, ethyl acetate, acetone and methanol, were distilled for the isolation process.

2.3. Instrumentation

Determination of the compound structures was carried out using spectrophotometer instruments. Additionally, ^{1}H and ^{13}C NMR were recorded using a Bruker AVANCE-300 Ultrashield NMR spectrometer at 300 and 75 MHz and AVANCE-500 at 500 and 125 MHz, respectively. Chemical shift values are shown as δ values with tetramethylsilane (TMS) as an internal reference. Peak multiplicities are quoted in Hz. Perkin-Elmer Lambda 35 UV-Vis was used to identify the UV spectra, while the IR spectra were measured using Perkin-Elmer FT-IR. Melting point was measured using Fisher Johns 'micro melting point

apparatus'. The absorbance of 96 wells of tested samples was measured using Gen-5 Microplate Reader (Synergy HT). Radioactivity was measured using a Tri-Carb 3110 TR PerkinElmer Liquid Scintillation Analyzer.

HRESI-MS was obtained via an Agilent 6530 Accurate-Mass Q-TOF LC/MS equipped with dual-ESI source. Agilent Technology, Zorbex Eclipse Plus C18 column, was used (1.8 μM particle size, 2.1 × 100 mm culomn dimension). The temperature was maintained at 40 °C. Mobile phase consists of (A) 0.1% formic acid (Supelco Inc., Pennsylvania, USA) in ultrapure water and (B) 0.1% formic acid in acetonitrile (LiChrosolv, Darmstadt, Germany) for +ve mode; (A) 0.1% ammonium formate in ultrapure water and (B) 100% acetonitrile for -ve mode. The flow rate was 0.25 mL/min. The injection volume was 2 μL. Data was stored at the mass range of m/z 50 to 1000.

Malaysianol F (**1**). Brown amorphous powder (6.2 mg), m.p 218–220 °C, HREITOFMS (positive mode) [M + H] +: m/z 696 (Calc. for C42H32O10). UV (MeOH) λmax nm: 200, 219, 288 and 340. IR (KBr) νmax cm^{-1}: 3384, 1621, 1468, 1163, 941, 803. ^{1}H NMR (300 MHz, acetone-d6): δH7.24 (2H, d, *J* = 8.7 Hz, H-2a/6a), 7.08 (^{1}H, d, *J* = 8.4 Hz, H-6c), 6.86 (2H, d, *J* = 8.7 Hz, H-2b/6b) 6.83 (2H, d, *J* = 8.7 Hz, H-3a/5a), 6.60 (2H, d, *J* = 8.7 Hz, H-3b/5b), 6.57 (2H, s, H-7b, 8b), 6.48 (^{1}H, s, H-12b), 6.45 (^{1}H, d, **J** = 2.4 Hz, H-3c), 6.33 (^{1}H, dd, *J* = 2.4, 7.5 Hz, H-5c), 6.30 (2H, d, *J* = 2.4 Hz, H-10c/14c), 6.23 (2H, d, *J* = 1.8 Hz, H-10a/14a), 6.22 (^{1}H, t, *J* = 1.8 Hz, H-12a), 6.19 (^{1}H, t, *J* = 2.4 Hz, H-12c), 4.53/5.77 (^{1}H each, d, *J* = 4.2, 3.9 Hz, H-7c/8c), 4.51/5.44 (^{1}H each, d, *J* = 5.4, 5.7 Hz, H-7a/8a). ^{13}C NMR (75 MHz, acetone-d6): 163.8 (C-13b), 163.3 (C-11b), 161.0 (C-11a/13a), 160.6 (C-11c/13c), 160.0 (C-4c), 159.3/159.2 (C-4b/4a), 157.3 (C-2c), 148.9 (C-9c), 148.2 (C-9a), 134.8 (C-7b), 134.1 (C-9b), 134.1 (C-1a), 129.5 (C-1b), 129.5 (C-2b/6b), 129.0 (C-2a/6a), 128.9 (C-6c), 123.4 (C-8b), 121.6 (C-1c), 121.5 (C-14b), 120.9 (C-10b), 117.2 (C-3a/5a), 117.1 (C-3b/5b), 108.3 (C-5c), 108.2 (C-10c/14c), 108.0 (C-10a/14a), 104.6 (C-3c), 103.1 (C-12c), 102.9 (C-12a), 95.1 (C-7a), 92.3 (C-12b), 90.5 (C-7c), 59.0 (C-8a), 57.5 (C-8c).

2.4. Extraction and Isolation

The ground, air-dried lianas of *G. microcarpum* (2 kg) were extracted using acetone (10 L) five times for three days at room temperature. The dried crude extract was then youdissolved in methanol and fractionated with diethyl ether to form ether-soluble and insoluble layers to remove tannins. The ether-soluble material was concentrated in vacuo at 40 °C to yield crude extract with less tannin. The tanninless crude extract (52.5 g) was fractionated using vacuum liquid chromatography (VLC) over silica gel, which was eluted with the mixtures of Hex: EtOAc with increasing polarity (from 7:3 to 0:10), followed by EtOAc: MeOH (9:1 and 8:2) to give five major fractions (GM1-GM5). Fraction GM2 (4.68 g) was chosen and subjected to further isolation and purification using VLC with Hex: EtOAc (from 9:1 to 0:10) and EtOAc: MeOH (9:1) as solvent systems to obtain six subfractions (GM21-GM26). Further purification of GM23 (900 mg) subfraction using repetitive radial chromatography technique with a solvent system of Hex: Acetone (8:2) yielded **3** (2.4 mg) and **11** (2.5 mg). Subfraction GM24 (1.50 g) was also subjected to repetitive radial chromatography technique to obtain **5** (6.5 mg) and **9** (3.2 mg). Fraction GM3 undergoes VLC with Hex: EtOAc (from 7:3 to 2:8) and EtOAc: MeOH (9:1) as solvent system to give 9 subfractions (GM31-GM39). GM31 was identified as **7** (12.2 mg). Repetitive radial chromatography ($CHCl_3$: Acetone (8:2)) on GM35 (317.4 mg) gave out **6** (3.5 mg) and **8** (8 mg). Meanwhile, GM36 (666.4 mg) was subjected to column chromatography containing Sephadex (100% MeOH as solvent system) to yield six fractions (GM361-GM366). GM364 (43.3 mg) was further purified via radial chromatography using $CHCl_3$: Acetone: MeOH (8.5:1:0.5) to obtain **4** (10 mg). Next, four subfractions were obtained (GM41-GM44) after VLC of fraction GM4 (9.29 g). Subfraction GM44 (1.29 g) was further purified via radial chromatography using $CHCl_3$: MeOH (8.5:1.5) as solvent system, yielding **10** (4.8 mg), **2** (62.5 mg) and **1** (6.2 mg). All of these compounds (**1**–**11**) were isolated for the first time from this plant. Their structures were determined on the basis of the analysis of spectral evidence by NMR data analyses. The ^{1}H and ^{13}C NMR spectral data for all compounds, together

with 2D NMR and CD spectra for malaysianol F (**1**) were provided in the Supplementary Material files. Compound **2** was previously reported in *Dryobalanops* plants [10] and had never been reported in *Gnetum* plants.

2.5. PGE_2 Inhibition Assay

The inhibition of PGE_2 production, indicated by the concentration of PGE_2 in human whole blood, was measured according to the validated radioimmunoassay (RIA) method [11]. The application of human blood was permitted by the Ethics Committee of Universiti Kebangsaan Malaysia (UKM), approval number NF-016-2013. Indomethacin was used as standard.

Human whole blood was drawn via aseptic vein puncture from healthy volunteers after two weeks without medicine or supplements and fasting for 8 h prior to blood being withdrawn. Moreover, 10% (*v*/*v*) of 2% EDTA was added to a polypropylene tube to prevent blood coagulation. Duplicates of 1 mL aliquots of EDTA-human whole blood samples were transferred into test tubes and incubated with 10 µL of sample or indomethacin for 15 min (37 °C) before the addition of LPS. The effects of samples or indomethacin on PGE_2 production were studied through the incubation of each sample with whole blood-EDTA in the presence of LPS (10 µg/mL in 0.9% normal saline) for 24 h. The concentration of samples was adjusted in five serial dilutions over a concentration range of 0.625 to 10 µg/mL for IC_{50}. After 24 h of incubation, the plasma was separated via centrifugation at 2600× *g* for 15 min at 4 °C. Triplicates of 100 µL aliquots were transferred into test tubes containing anti-PGE_2 (100 µL; diluted with a ratio of 1:50,000) and [^{3}H]-PGE_2 (100 µg/mL; 5000 cpm) and incubated for 18–24 h at 4 °C. Dextran-charcoal solution (200 µL) was added to the mixture and once again incubated for 10 min at 0 °C. Centrifugation at 3000× *g* for 15 min at 4 °C was carried out to separate the supernatants, which were pipetted (300 µL) into a liquid scintillation cocktail (3 mL). The radioactivity was measured using a liquid scintillation analyzer. The inhibition rates were calculated using the following formula:

$$\% \text{ inhibition} = (1 - [PGE_2 \text{ in samples or indomethacin}]/[PGE_2 \text{ in negative control}]) \times 100 \quad (1)$$

3. Results and Discussion

3.1. Phytochemicals from Lianas G. microcarpum

The phytochemical investigation of the acetone extract of the lianas of *G. microcarpum* yielded eleven stilbenes, including one new compound identified as malaysianol F (**1**). Based on the comparison of ^{1}H and ^{13}C NMR and physicochemical data with those reported in the literature, the known compounds were identified as malaysianol D (**2**) [10], malaysianol E (**3**) [12], ε- viniferin (**4**) [13], resveratrol (**5**) [14], gnetol (**6**) [15], gnetucleistol C (**7**) [16], isorhapontigenin (**8**) [17], cuspidan B (**9**) [18], parvifolol D (**10**) [19] and gnetifolin M (**11**) [20].

Compound **1** was isolated as a brown amorphous powder. The pure compound underwent analysis using the LC-Mass spectrum (HR-ESI-TOF-MS), which revealed the presence of a prominent protonated molecule ion with an *m*/*z* value that showed an $[M + H]^+$ ion peak at *m*/*z* 697.2093 corresponding to the molecular formula of $C_{42}H_{32}O_{10}$, indicating a trimer. The ^{13}C APT NMR exhibited 35 signals representing 42 carbons. The spectrum showed that **1** was formed by 18 aromatic methine carbons (δ_C92.3–129.5), eight quaternary aromatic carbons (δ_C120.9–148.9) and 10 oxyaryl carbons (δ_C157.3–163.8). Additionally, there were two pairs of aliphatic methine carbons (δ_C57.5 and δ_C59.0) and aliphatic oxymethine carbons (δ_C90.5 and δ_C95.1) observed, which revealed the existence of two furan rings in the structure. In addition, a pair of olefinic methine carbons (δ_C123.4 and δ_C134.8), representing a free stilbene skeleton, was also shown. This fact further supported the molecular formula of $C_{42}H_{32}O_{10}$ suggested by the mass spectral data.

The ^{1}H NMR spectrum revealed the presence of four sets of *ortho*-coupled aromatic methine protons in an AABB spin system at δ_H6.60/δ_H6.86 (2H each, d, *J* = 8.7 Hz, H-3b/5b, H-2b/6b) and δ_H6.83/δ_H7.24 (2H each, d, *J* = 8.7 Hz, H-3a/5a, H-2a/6a) belonging to two

units of the *p*-hydroxybenzene ring. Signals of four sets of *meta*-coupled aromatic methine protons were observed at δ_H6.22/6.23 (1H, t, *J* = 1.8 Hz/2H, d, *J* = 1.8 Hz, H-10a/14a, H-12a) and δ_H6.19/6.30 (1H, t, *J* = 2.4 Hz/2H, d, *J* = 2.4 Hz, H-10c/14c, H-12c) in an AB_2 spin system attributable to two units of 1, 3, 5-trisubstituted benzene rings. A singlet was observed at δ_H6.48 (1H, s, H-12b), assignable to a unit of 3, 5-dihydroxylbenzene ring. Then, two pairs of mutually coupled aliphatic methine and oxymethine protons at δ_H4.51/5.44 (1H each, d, *J* = 5.4, 5.7 Hz, H-7a/8a) and δ_H4.53/5.77 (1H each, d, *J* = 4.2, 3.9 Hz, H-7c/8c) showed the existence of two units of 1, 2-dihydrobenzofuran moiety. In addition, a pair of *trans*-olefinic protons, which appeared as a singlet at δ_H6.57 (2H), was dedicated to a 1, 2-disubstituted vinyl group. Finally, three sets of aromatic methine protons in an ABD spin system resonated at δ_H6.30 (1H, dd, *J* = 2.4, 7.5 Hz, H-5c), 6.45 (1H, d, *J* = 2.4 Hz, H-3c) and 7.08 (1H, d, *J* = 8.4 Hz, H-6c), showing one unit of 1, 2, 4-trisubstituted benzene ring.

The HMBC spectrum of **1** (Figure 2) was observed to further confirm the structure. The spectrum displayed long-range correlations between aromatic methine protons at δ_H7.24 (H-2a/6a) with oxymethine aliphatic carbon C-7a and aromatic methine proton at δ_H6.23 (H-10a/14a) with aliphatic carbon C-8a confirming the attachment of ring A1 and A2 at C-7a and C-8a. The same situation can be observed for the attachment of ring B1 and B2 at C-7b and C-8b as correlations occurred between the olefinic methine proton at δ_H6.57 (H-7b/8b) with quaternary carbon C-14b and C-9b and with the aromatic methine carbon C-2b/6b). In addition, the long-range correlations between the aromatic proton at δ_H6.30 (H-10c/14c) with C-8c and the aromatic methine proton at δ_H7.08 (H-6c) with C-7c suggests the attachment of ring C-1 and C-2 at C-8c and C-7c, respectively. The spectrum also showed correlations between aliphatic protons H-7c/8c with aromatic carbons C-14b/C-13b, disclosing the formation of a benzofuran ring from the condensation of C-7c/C-8c with the aromatic carbons of ring B2. The planar structure of **1** was concluded, as shown in Figure 1.

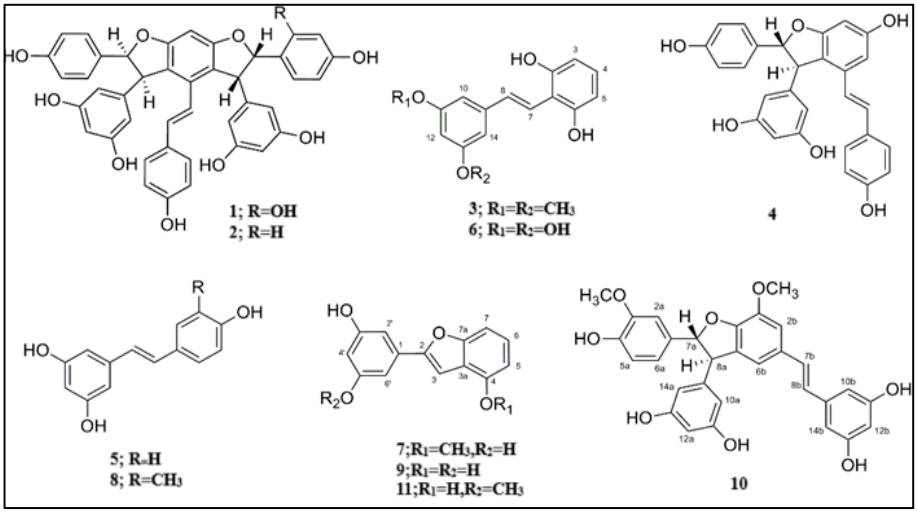

Figure 1. Chemical structures of compounds **1**–**11** isolated from *G. microcarpum*.

Meanwhile, the NOESY spectrum of **1** showed correlations similar to that of the COSY spectrum, with the exception of the correlation between H-7c and H-8c. The relative stereochemistry of H-7a/8a was determined to be in *cis*-orientation since the correlation observed in NOE showed that the two atoms had the same direction, while the stereochemistry of H-7c/8c could not be determined due to the lack of sample, which resulted in the absence of any correlations in the NOESY spectrum (Figure 2). Nevertheless, the ECD experiment was carried out on **1** for the assignment of its absolute configuration, and the spectrum was compared with that of **2.** The CD spectrum of **1** showed the same pattern as **2**, implying that **1** has the same (7a*S*,8a*R*/7c*S*,8c*R*) absolute configuration. Based on this, stereochemistry at

H-7c/8c of **1** can be determined as *cis*-oriented. According to the above-analyzed data and discussion, **1** was determined to be malaysianol F, a new hydroxylated resveratrol trimer.

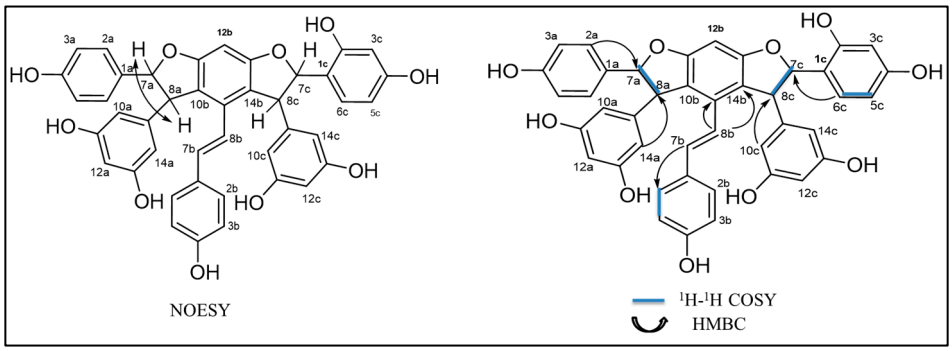

Figure 2. Important COSY, HMBC and NOE correlations of malaysianol F.

The plausible biosynthesis of trimer malaysianol F (**1**) presented in Figure 3 can be suggested from the oxidative coupling reaction of resveratrol radical with an active site at C-8c and ε-viniferin radical with an active site at C-14b [10]. As the bonding formed, the formation of a benzofuran ring takes place via an intermolecular cyclization reaction in acidic conditions, which used the oxygen of O-13b and olefinic carbon C-7c to obtain the structure of malaysianol D (**2**). Malaysianol F (**1**) was then formed via direct hydroxylation of malaysianol D (**2**) at C-2c.

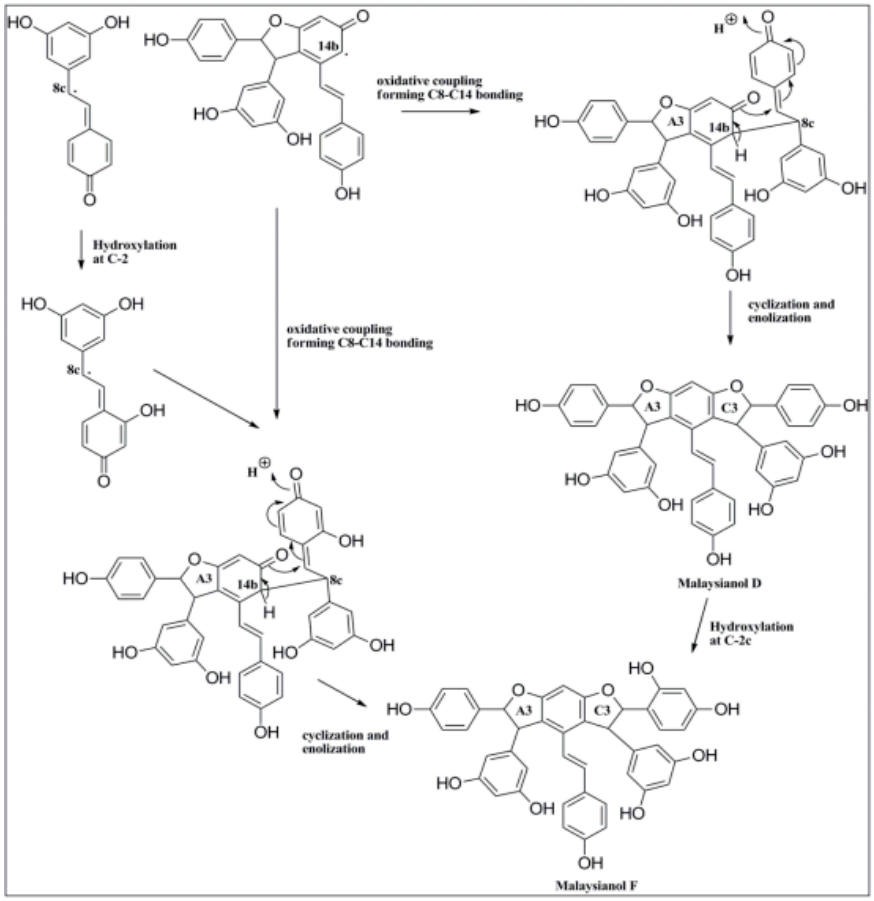

Figure 3. Proposed biosynthesis of malaysianol F.

3.2. PGE_2 Inhibitory Activities

Five of the isolated stilbenoids (**2**, **4**, **5**, **6** and **8**) were subjected to radioimmunoassay tests and were able to inhibit the PGE_2 production induced by LPS with a percentage inhibition in the range of 43.05% to 74.86% at a concentration of 10 g/mL. Based on the result displayed in Table 1, the monomeric stilbene, together with one dimeric stilbenoid, displayed anti-inflammatory properties. Resveratrol (**5**) has long been known to exert anti-inflammatory properties. Similarly, in this study, **5** exhibited 62.88% of PGE_2 inhibitory activity at 10 µg/mL. Gnetol (**6**) exhibited a slightly higher activity compared to **5** (72.68%), and isorhapontigenin (**8**) possessed the highest activity (74.86%). These three stilbenoids have the structure of a basic stilbene skeleton. The dimer ε-viniferin (**4**) showed moderate activity (52.70%). Finally, malaysianol D (**2**), the stilbene trimer, exhibited only a weak PGE_2 inhibitory activity (43.05%). Both oligostilbenes **2** and **4** possess a benzofuran ring in their structure. Compound **6** was identified as a promising inhibitor, with an IC_{50} of 1.84 M comparable to indomethachin, which has an IC_{50} of 1.29 M.

Table 1. % Inhibition and IC_{50} values of PGE_2 production by LPS-stimulated human blood.

Sample	PGE_2 Inhibition	
	% Inhibition	**IC_{50}**
2	43.05	Nd
4	52.70	Nd
5	62.88	6.57
6	72.68	1.84
8	74.86	3.68
Indomethacin	85.29	1.29

3.3. Structure–Activity Relationship Study

There are limited SAR studies on the PGE_2 inhibitory activity of stilbene compounds, specifically oligostilbenes. Previous studies on the structure–activity relationship of stilbenes have revealed that increasing the number of hydroxyl groups at their *ortho* position on the phenol ring could increase the free radical scavenging capacity, the cytotoxic activity and the anti-inflammatory effects of these compounds. Nevertheless, manipulation of the stilbenes structure can improve its bioavailability and activity [21]. An example can be seen in animal studies, where 3,4,5,4′-tetramethoxystilbene (DMU-212), by blocking the C4-OH by methylation, shows stronger antiproliferative properties in human colon cancer cells than resveratrol, possibly, because these methylated groups could provide better plasma levels by slowing excretion [22]. Another example, pinnosylvin, which is more lipophilic as it lacks one hydroxyl at C-4′, was found to inhibit the COX-2-mediated PGE_2 production better than resveratrol [23].

Results on PGE2 inhibitory activity showed that, among the stilbenoid compounds tested in this study, all the stilbene monomers, together with one dimeric stilbene, displayed anti-inflammatory properties. Resveratrol (**5**), specifically, has long been known to exert anti-inflammatory properties and has been proven to be a non-selective inhibitor of COX-1 and COX-2 [24]. Similarly, in this study, **5** exhibited good PGE_2 inhibitory activity with an inhibition % of 62.88 (IC_{50} of 6.57 µM) at a concentration of 10 ug/mL. Nonetheless, isorhapontigenin (**8**) exhibited higher activity compared to resveratrol (74.86%), followed by gnetol (**6**) (72.68%) at the same concentration. All these three stilbenoid monomers have the structure of a basic stilbene skeleton. Based on these results, the increase in the number of hydroxyl groups performed better, as previously proven [25]. On the other hand, the presence of the methoxy group at the *ortho*-position in the structure of **8** also seems to have a positive effect on the reactivity. Similarly, one study has shown the dimethoxystilbene compound as a potent PGE_2 inhibitor [23]. The stilbene dimer ε-viniferin (**4**) showed moderate activity (52.70% at 10 µg/mL), while malaysianol D (**2**), the stilbene trimer, also exhibited moderate PGE_2 inhibitory activity (43.05%) at the same concentration. According

to these results, it can be suggested that the benzofuran ring does not have a significant effect ono the activity as both **2** and **4** possess benzofuran rings in their structure. Based on the observation, the addition of a methoxy group, specifically in the *ortho*-position of stilbenes, such as in **8**, causes significant activity [21]. The methoxy gave higher lipophilicity to the compounds, which may favor their entry into cells and confer more resistance to degradation, thus improving pharmacokinetics [21]. However, the number of methoxy and hydroxyl groups must be in equilibrium, as an excessive number of methoxylated groups may impair the interaction with the target protein [26]. The hydroxyl group confers more solubility, which allows for a better interaction with proteins [27], whereas the methoxylated group confer resistance to degradation. Nevertheless, in some cases, the hydroxyl groups still contribute to the activity of stilbenes, as can be seen in gnetol (**6**), which has an additional OH in its structure and exerts a higher activity compared to **5**. As a result, this study demonstrated the importance of the methoxy group, specifically in the *o*-position, followed by the number of hydroxyl groups in stilbenes and oligostilbenes structure for their PGE_2 inhibitory activity. Furthermore, the benzofuran ring in oligostilbenes does not have a significant effect on PGE_2 inhibitory activity. Above all, gnetol (**6**) exerted significant concentration-dependent inhibitory effects on PGE_2 production with IC_{50} values of 1.84 μM comparable with the positive control, indomethacin (IC_{50} 1.29 μM).

4. Conclusions

The phytochemical and anti-inflammatory properties of the lianas of *Gnetum microcarpum* Blume have been investigated. One new compound, malaysianol F (**1**) and ten known compounds, resveratrol (**5**), isorhapontigenin (**8**), gnetol (**6**), gnetuclesitol C (**7**), cuspidan B (**9**), ε-viniferin (**4**), parvifolol D (**10**), gnemonol M (**11**) and malaysianol D (**2**), were successfully obtained. All compounds were isolated for the first time in *G. microcarpum,* while 8 and 9 have been previously reported in *G. cuspidatum*. The new oligostilbene, malaysianol F (**1**), was formed via the hydroxylation of malaysianol D (**2**) at C-2. Based on the result, the three monomeric stilbenes, 5, 6 and 8, together with one dimeric stilbenoid 4, displayed anti-inflammation properties. The monomer stilbenoids have a basic stilbene skeleton structure. In this case, the hydroxyl groups contribute to the activity of stilbenes, such as in **6,** which has an additional OH in its structure and exerts a higher activity compared to **5**. However, the presence of the methoxy group at the *ortho*-position in structure **8** resulted in even higher activity. The dimer ε-viniferin (**4**) showed moderate activity and malaysianol D (**2**), and the stilbene trimer exhibited only weak PGE2-inhibitory activity. According to the results, the benzofuran ring does not have a significant effect on the activity as both **4** and **2** possess benzofuran rings in their structure. An increase in the size of the stilbenoid compounds was also seen to have an effect on the activity as bigger molecules (**2**) have a lower activity. In addition, gnetol (**6**) was identified as a possible new drug candidate for PGE2 inhibition.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations10090496/s1, Figure S1: The HREIMS chromatogram for compound **1**; Figure S2: The IR spectrum of compound **1**; Figure S3: ^{13}C APT spectrum of compound **1**; Figure S4: ^{1}H NMR spectrum of compound **1**; Figure S5: COSY spectrum of compound **1**; Figure S6: HMBC spectrum of compound **1**; Figure S7: NOESY spectrum of compound **1**; Figure S8: CD spectrum of malaysianol D (1) and F (2); Figure S9: TLC chromatogram of *G. microcarpum* after VLC; Figure S10: ^{1}H and ^{13}C NMR spectra of malaysianol E; Figure S11: ^{1}H and ^{13}C NMR spectra of gnetol; Figure S12: ^{1}H and ^{13}C NMR spectra of resveratrol; Figure S13: ^{1}H and ^{13}C NMR spectra of gnetucleistol c; Figure S14: ^{1}H and ^{13}C NMR spectra of e-viniferin; Figure S15: ^{1}H and ^{13}C NMR spectra of parvifolol d; Figure S16: ^{1}H and ^{13}C NMR spectra of malaysianol D; Figure S17: ^{1}H and ^{13}C NMR spectra of cuspidan B; Figure S18: ^{1}H and ^{13}C NMR spectra of isorhapontigenin; Figure S19: ^{1}H and ^{13}C NMR spectra of gnetifolin m.

Author Contributions: Conceptualization, N.F.N.A. and N.A.; methodology, N.F.N.A., C.W.S., N.K.N.A.Z., S.N.M.S. and N.A.; software, M.I.M.Y.; validation, N.A., J.J. and M.T.; formal analysis, N.F.N.A. and N.A.; investigation, N.F.N.A.; resources, N.A.; writing—original draft preparation, N.F.N.A.; writing—review and editing, N.F.N.A., A.S.K. and N.A.; visualization, N.F.N.A.; supervision, N.A.; project administration, N.A.; funding acquisition, N.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by FRGS/1/2021/STG04/UITM/01/1, Fundamental Research Grant Scheme, Ministry of Higher Education, Malaysia.

Acknowledgments: The authors would like to thank the Faculty of Applied Sciences, Universiti Teknologi MARA, and the Faculty of Pharmacy, Universiti Kebangsaan Malaysia, for the laboratory space and facilities.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ali, M.I.; Shabir, S.; Soni, L.K.; Dobhal, M.P.; Moin, S. The Phytochemical Potential of Gnetaceae with Peculiar Reference to *Gnetum ula* and Traditional Uses of *Gnetaceae* Species. *Plant Arch.* **2020**, *20*, 2979–2986.
2. Hou, C.; Humphreys, A.M.; Thureborn, O.; Rydin, C. New insights into the evolutionary history of *Gnetum* (Gnetales). *Taxon.* **2015**, *64*, 239–253. [CrossRef]
3. Reddy, S.M.; Chary, S.J. *University Botany II: (Gymnosperms, Plant Anatomy, Genetics, Ecology)*; New Age International: Delhi, India, 2003; Volume 2.
4. Bhatnagar, S.P.; Moitra, A. *Gymnosperms*; New Age International: Delhi, India, 1996.
5. Eisai, P.T. *Medicinal Herb Index in Indonesia*; PT Eisai Indonesia: Jakarta, Indonesia, 1995; Volume 91.
6. Sotheeswaran, S.; Pasupathy, V. Distribution of resveratrol oligomers in plants. *Phytochemistry* **1993**, *32*, 1083–1092. [CrossRef]
7. Xue, Y.Q.; Di, J.M.; Luo, Y.; Cheng, K.J.; Wei, X.; Shi, Z. Resveratrol oligomers for the prevention and treatment of cancers. *Oxidative Med. Cell. Longev.* **2014**, *2014*, 765832. [CrossRef]
8. Govaerts, R. *World Checklist of Seed Plants*; MIM: Antwerp Belgium, 1995.
9. Baloch, E. *Gnetum Microcarpum*; The IUCN Red List of Threatened Species: London, UK, 2013.
10. Wibowo, A.; Ahmat, N.; Hamzah, A.S.; Latif, F.A.; Norrizah, J.S.; Khong, H.Y.; Takayama, H. Identification and biological activity of secondary metabolites from *Dryobalanops beccarii*. *Phytochem. Lett.* **2014**, *9*, 117–122. [CrossRef]
11. Patrignani, P.; Panara, M.R.; Greco, A.; Fusco, O.; Natoli, C.; Iacobelli, S.; Cipollone, F.; Ganci, A.; Créminon, C.; Maclouf, J.; et al. Biochemical and pharmacological characterization of the cyclooxygenase activity of human blood prostaglandin endoperoxide synthases. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1705–1712.
12. Azmin, N.F.N.; Ahmat, N.; Syah, Y.M.; Zawawi, N.K.N.; Yusof, M.I.M. A new stilbenoid compound from the lianas of *Gnetum microcarpum*. *Nat. Prod. Commun.* **2014**, *9*, 1743–1744. [CrossRef]
13. Oshima, Y.; Kamijou, A.; Ohizumi, Y.; Niwa, M.; Ito, J.; Hisamichi, K.; Takeshita, M. Novel oligostilbenes from *Vitis coignetiae*. *Tetrahedron* **1995**, *51*, 11979–11986. [CrossRef]
14. Zaman, A.; Prakash, S.; Wizarat, K.; Joshi, B.S.; Gawad, O.H.; Likhate, K. Isolation and structure of gnetol, a novel stilbene from *Gnetum ula*. *Indian J. Chem.* **1983**, *23B*, 101–104.
15. Mattivi, F.; Reneiro, F.; Korhammer, S. Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. *J. Agric. Food Chem.* **2005**, *43*, 1820–1823. [CrossRef]
16. Yao, C.S.; Lin, M.; Liu, X.; Wang, Y. Stilbenes from *Gnetum cleistostachyum*. *Huaxue Xuebao* **2003**, *61*, 1331–1334.
17. Fernández-Marín, M.I.; Guerrero, R.F.; García-Parrilla, M.C.; Puertas, B.; Richard, T.; Rodriguez-Werner, M.A.; Winterhalter, P.; Monti, J.P.; Cantos-Villar, E. Isorhapontigenin: A novel bioactive stilbene from wine grapes. *Food Chem.* **2012**, *135*, 1353–1359. [CrossRef] [PubMed]
18. Shimokawa, Y.; Hirasawa, Y.; Kaneda, T.; Hadi, A.H.A.; Morita, H. Cuspidans A and B, Two New Stilbenoids from the Bark of *Gnetum cuspidatum*. *Chem. Pharm. Bull.* **2012**, *60*, 790–792. [CrossRef] [PubMed]
19. Xiang, W.; Jiang, B.; Li, X.; Zhang, H. Constituents of *Gnetum montanum*. *Fitoterapia* **2002**, *73*, 40–42. [CrossRef]
20. Tanaka, T.; Iliya, I.; Ito, T.; Furusawa, M.; Nakaya, K.; Iinuma, M.; Shirataki, Y.; Matsuura, N.; Ubukata, M.; Murata, J.; et al. Stilbenoids in Lianas of *Gnetum parvifolium*. *Chem. Pharm. Bull.* **2001**, *49*, 858–862. [CrossRef]
21. Sirerol, J.A.; Rodríguez, M.L.; Mena, S.; Asensi, M.A.; Estrela, J.M.; Ortega, A.L. Role of natural stilbenes in the prevention of cancer. *Oxidative Med. Cell. Longev.* **2016**, *2016*, 3128951. [CrossRef]
22. Sale, S.; Tunstall, R.G.; Ruparelia, K.C.; Potter, G.A.; Steward, W.P.; Gescher, A.J. Comparison of the effects of the chemopreventive agent resveratrol and its synthetic analog trans 3, 4, 5, 4′-tetramethoxystilbene (DMU-212) on adenoma development in the ApcMin+ mouse and cyclooxygenase-2 in human-derived colon cancer cells. *Int. J. Cancer* **2005**, *115*, 194–201. [CrossRef]
23. Park, E.; Min, H.; Ahn, Y.; Bae, C.; Pyee, J.; Lee, S. Synthesis and inhibitory effects of pinosylvin derivatives on prostaglandin E2 production in lipopolysaccharide-induced mouse macrophage cells. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5895–5898. [CrossRef]

24. Likhitwitayawuid, K.; Sawasdee, K.; Kirtikara, K. Flavonoids and Stilbenoids with COX-1 and COX-2 Inhibitory Activity from *Dracaena loureiri*. *Planta Med.* **2002**, *68*, 841–843. [CrossRef]
25. Murias, M.; Handler, N.; Erker, T.; Pleban, K.; Ecker, G.; Saiko, P.; Ja, W. Resveratrol analogues as selective cyclooxygenase-2 inhibitors: Synthesis and structure—Activity relationship. *Bioorg. Med. Chem.* **2004**, *12*, 5571–5578. [CrossRef]
26. Shi, Y.W.; Wang, C.P.; Liu, L.; Liu, Y.L.; Wang, X.; Hong, Y.; Kong, L.D. Antihyperuricemic and nephroprotective effects of resveratrol and its analogues in hyperuricemic mice. *Mol. Nutr. Food Res.* **2012**, *56*, 1433–1444. [CrossRef] [PubMed]
27. Bohn, T. Dietary factors affecting polyphenol bioavailability. *Nutr. Rev.* **2014**, *72*, 429–452. [CrossRef] [PubMed]