RESVERATROLS FROM THE STEMBARK OF *SHOREA OVALIS* (MERANTI KEPONG) (DIPTEROCARPACEAE) AND ANTIOXIDANT ACTIVITY

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Abstract

Dipterocarpaceae is a plant family of large tree that is widely distributed in the tropical rain forest of Southeast Asia, such as Borneo and Peninsular Malaysia. *Shorea* is the largest genus of Dipterocarpaceae and the most economically important in Malaysia. Locally, *Shorea* is known as Balau, Meranti Pa'ang, Meranti Damar Hitam and Red Meranti. *Shorea ovalis* is the species included in the group of Red Meranti. Locally it is known as Meranti Kepong. Currently, very limited chemical constituents have been reported from this species. In this research, a phytochemical study was conducted on the stem bark of *Shorea ovalis*. The powder of the cleaned, chopped and grinded stem-bark was extracted using acetone *via* maceration. The isolation and purification of the compound were carried out using vacuum liquid chromatography followed by radial chromatography and preparative thin layer chromatography. The structure of the pure compounds was elucidated by means of spectroscopic methods including NMR, UV- Vis, FTIR and comparison with literature. Two compounds were isolated and identified as resveratrol and α-viniferin. Resveratrol was reported for the first time for this plant. α-viniferin that has been previously reported, was subjected to antioxidant activity. However, this compound did not show significant antioxidant property.

Keywords: antioxidant, Dipterocarpaceae, resveratrol, *Shorea ovalis*, α-viniferin.

Introduction

Malaysia is known with a tropical rainforest that rich in diverse ecosystem. From the large number of diverse types of plants, Dipterocarpaceae and Euphorbiaceae family is among the largest group of plant. Dipterocarpaceae is the most important family of timber producing trees in this country. This family is the dominant family in the forests in terms of volume. This family consists of three subfamilies: Pakaraimoideae (one genus, one species), Monotoideae (three genera, about 40 species) and Dipterocarpoideae (13 genera, about 475 species) (Appanah & Turnbull, 1998). In Malaysian rainforests, the dipterocarps is the most abundant, whereas in some areas, it covers 80 % of the canopy trees (Ashton, 1982). The largest genera are *Shorea* (196 species) followed by *Hopea* (104 species), *Dipterocarpus* (70

species) and *Vatica* (65 speices) (Appanah & Turnbull, 1998). Almost all the species of this family are of commercial importance (Wong, 2002).

Shorea is the largest and economical important genus of Dipterocarpaceae. It is found growing in Peninsular Malaysia (59 species), Sumatra and Borneo. *Shorea* is locally known as Balau, Meranti Pa'ang, Meranti Dammar Hitam and Red Meranti (Cao, 2006). Generally, the wood of *Shorea* is soft and light, with colour usually pink, red and yellow. There is also quite a variation in weight and hardness. The wood of *Shorea* is used for planks, temporary construction, packing cases, furniture, and many things, while, the Damar is used for fuel torch, varnish glues, medicine for diarrhea, skin diseases, dysentery and ear troubles. The oil, which is known as tengkawang oil is used as medicine for skin and mouth diseases (Rohaiza, 2011). *Shorea ovalis* is one of the species in the group of Red Meranti. In Malaysia, it's known as Meranti kepong (Peninsular Malaysia), Seraya kepong (Sabah) and Kepong sluang (Pahang) [3]. There is limited studies were done on *Shorea ovalis*, but from the previous study by Noviany and Setupo Hadi, (Noviany, 2009) from University of Lampung, Indonesia , they discovered the oligomeric stilbene and tetramer stilbene which include α-viniferin and hopeophenol extracted from the bark of this species. There are still a lot of phytochemical constituents need to be isolated and identified. Moreover, bioactivity of the compound from this species has never been reported.

The destruction of tropical rainforests threatens the survival of tropical plants and without proper documentation and study, the knowledge of the traditional uses of this plant might be lost forever. Thus, extensive phytochemical and biological assessments to this plant species are of utmost importance to preserve the knowledge of our natural heritage for the next generation. This study was carried out on the stem barks of *S. ovalis*. Up to this study was started, only two compounds have been reported and no biological property has been studied. The study of *Shorea ovalis* will provide more information for a future research as it will increase a database based on that genus and species. Thus, the isolated compounds in this species will be useful for drug development and medicine properties.

Material and Methods

General

The NMR spectra were recorded on a Bruker AV300 NMR spectrometer 1D-NMR (^{1}H-NMR, ^{13}C-APT) with TMS as internal standard. $CDCI_3$ and acetone-D as solvent. Silica gel 60 (Merck catalog number:1.00747) was used for vacuum liquid chromatography, Silica gel 60 PF254 Gypsum (Merck catalog number: 1.07749) for radial chromatography, Silica gel G_{60} 230-400 mesh (Merck catalog number: 1.09385) for column chromatography, TLC plates, Kieselgel 60 F_{254} of 1, 0.5 and 0.25 mm (Merck 5554) and visualized under UV light and by spraying with Cerium sulphate reagent followed by heating.

Plant collection

Fresh stem bark was collected from Hutan Simpan Jengka, Pahang. The stem bark was cleaned, chopped and dried under shade at room temperature until completely dry. Then, it was ground to coarse powder using mechanical grinder.

Extraction and isolation

The ground stem bark was extracted with acetone for 24 hours in a container covered by aluminum foil. It was filtered and evaporated to produce crude extract. This process was repeated several time and the crude extracts were combined to give 207.6 g crude acetone

extract. The crude was further processed to remove tannin. The crude was initially re-dissolved in 200 ml methanol, added with huge quantity of diethyl ether and shaken vigorously. It was left to settle down the tannin. The solution was then filtered and evaporated to give crude with reduced tannin content, with the mass 88.0 g of the crude acetone extract. To confirm if the tannin content was excluded from the extract by evaluating the extact on the TLC plate. The spot of tannin was observed to be disappeared. The reduced-tannin crude extract was subjected to vacuum liquid chromatography (VLC) with column of 10 or 15 cm.

The extract which contain less tannin was fractionated by VLC eluted with hexane: ethyl acetate (50:50 to 10:90) followed by ethyl acetate: methanol (90:10 to 80:20). The fractionation produced nine major fractions S1-S9 (7.2, 6.9, 1.7, 6.1, 11.0, 7.0, 13.6, 3.3, 32.1 g respectively). Fraction S4 was refractionated by VLC (eluent, chloroform: ethyl acetate: methanol 6:3.5:0.5 gave five fractions (S41-S44). Fraction S42 was further refractionated with radial chromatography (RC) using eluent, chloroform: methanol (95:5) yielded S421-S428. Purification of fraction S426 with RC (eluent, chloroform:methanol 9.5:5) produced compound **1** (21.2 mg). Fraction S7 also refractionated using VLC with eluent, chloroform: ethyl acetate: acetone (85:10:5). This yielded eleven fractions S71-S711. The fraction S78 was further isolated by RC (eluent, chloroform: ethyl acetate: methanol 60:35:5 to give 8 fractions S781-S788. Furhter refractionation of S783 using RC (eluent, chloroform: ethyl acetate 95:5 to 80:20) gave six fractions; S7831-S7836. Finally, purification of fraction S7831 with preparative thin layer chromatography (PTLC) (eluent, chloroform:methanol 95:5) yielded compound **2** (1.3 mg).

The compounds were detected by TLC profile and confirmed by $CeSO_4$ reagent. The compounds were characterized using NMR, and comparison with previous data to confirm that compound 1 is α-viniferin and compound 2 is resveratrol.

DPPH free radical scavenging activity

The DPPH free radical scavenging assay was performed on α-viniferin using a 96-well microtiter plate. The stock solution of the ferulic acid derivatives was prepared at 1 mg/ml concentration in methanol and diluted to 200 mg/ml (the substock solution). The substock solution was introduced into respective microtitre wells in a successfully descending amount of 100, 80, 60, 20, 10 and 5 µl. The stock solution in each well was then diluted with methanol to make the total volume 195 µl, and finally added with 5 µl of DPPH solution (prepared at 1 mg/ml in methanol). The plate was shaken to ensure thorough mixing before placing it in the dark. After allowing it to stand for 30 minutes, the optical density of the solution was read using a Gen-5 Microplate Reader at wavelength 517 nm. The radical scavenging activity of the tested compounds was calculated according to the Eq. (1), which was expressed as percentage of DPPH inhibition. Inhibition (%) versus concentration (µ/ml) curve was plotted, and express as IC_{50} value using a non-linear regression algorithm. The data were presented as mean values ± standard deviation (n=3). A_A and A_B are the absorbance values of the blank (DPPH) and test samples (DPPH+sample) respectively.

$$\% \text{ DPPH inhibition} = (A_B - A_A) / A_A \quad (1)$$

The IC_{50} values denote the concentration of each sample required to give 50% of the absorbance shown by the control.

Result and discussion

In this study, a monomer and trimer stilbenoids have been isolated namely resveratrol and α-viniferin. **Compound (1)** α-viniferin as shown in Figure 1 was isolated as pale yellow powder. IR (in KBr pellet) spectrum showed several functional group characteristic; hydroxyl group at ν_{max} 3375 cm^{-1}, aromatic group at 1613, 1514, 1363 and 1220 cm^{-1}, and 827 cm^{-1} for *p*-disubstituted benzene (Noviany 2009). The ^{13}C NMR spectral data indicated the presence of 35 carbon signals representing 42 carbons suggesting a trimer oligostilbenoid type of compound (3 units x 14 carbons). The typical of trimer skeleton was supported by the presence of nine oxyaryl carbon indications (C-4a, 4b, 4c, 11a, 11b, 11c, 13a, 13b, 13c) at δ_C 158.9 – 162.8 and nine aromatic quartenary carbon (C-1a, 1b, 1c, 9a, 9b, 9c, 10a, 10b, 10c) at δ_C 119.8 – 142.2. Twelve aromatic methine carbon signals (C-2a/6a, 2b/6b, 2c/6c, 3a/5a, 3b/5b, 3c/5c) at δ_C 116.7-129.7 and (C-12a, 12b, 12c, 14a, 14b, 14c) at δ_C 97.6 – 109.6 for *p*-hydroxybenzene and disubstituted-3,5 dihydroxybenzene ring respectively. The occurrence of three oxymethine carbon signals (C-7a, 7b, 7c) at δ_C 87.4 – 96.6 and δ_C 47.4 – 56.7 indicated the existence of three aliphatic methine (C-8a, 8b, 8c) in the skeleton of compound 1. The ^1H NMR displayed the presence of six set of *ortho*-coupled aromatic proton signals assignable to three units of *p*-hydroxybenzene ring (H-2a/6a, 2b/6b, 2c/6c, H-3a/5a, 3b/5b, 3c/5c) at δ_H 7.04/6.73, 7.23/6.79 and 7.06/6.80, and six sets of *meta*-coupled aromatic protons with integration ^1H characteristic for three units of disubstituted-2,3-dihydroxybenzene ring (H-12a, 12b, 12c, H-14a, 14b, 14c) at δ_H 6.01/6.23, 6.74/6.25 and 6.61/6.27. The proton spectrum also showed the presence of three sets of aliphatic methine proton signals (H-7a, 7b, 7c, H-8a, 8b, 8c) at δ_H 4.92/4.62, 5.96/4.71 and 6.08/3.98 which supported the suggestion of the presence of three units of 1,2-dihydroxybenzofuran ring in the skeleton of α-viniferin as compared with the previous data reported by Tanaka et al (2000) and Kitanaka et al (1990). The ^1H and ^{13}C NMR data are shown in **Table 1**.

Table 1 The NMR spectral data of Compound 1 in acetone-d at 300 MHz (^1H) and 75 MHz (^{13}C)

No	Compound 1		α-viniferin *		α-viniferin **	
	δC	δH (J in Hz)	δC	δH (J in Hz)	δC	δH (J in Hz)
1a	133.0	-	132.0	-	131.5	-
2a/6a	129.2	7.04 (d, 8.4)	128.1	7.03 (d, 8.5)	127.4	7.04 (d, 8.8)
3a/5a	116.7	6.73 (d, 8.7)	115.7	6.72 (d, 8.5)	111.5	6.73 (d, 8.8)
4a	158.9	-	157.8	-	157.3	-
7a	87.4	6.08 (brs)	86.4	6.07 (brs)	85.9	6.08 (s)
8a	47.4	3.98 (brs)	46.4	3.97 (brs)	45.9	3.97 (s)
9a	142.2	-	141.2	-	139.2	-
10a	119.8	-	118.8	-	120.4	-
11a	160.4	-	159.3	-	158.8	-
12a	109.6	6.01 (d, 2.1)	108.5	5.99 (d, 1.8)	96.	6.26 (d, 2.0)
13a	162.6	-	161.6	-	158.9	-
14a	99.0	6.23 (d, 2.1)	98.0	6.22 (d, 1.8)	105.7	6.73 (d, 2.0)
1b	133.3	-	132.2	-	132.0	-
2b/6b	129.1	7.23 (d, 8.7)	128.1	7.22 (d, 8.5)	127.7	7.22 (d, 8.8)
3b/5b	117.1	6.79 (d, 8.4)	116.1	6.77 (d, 8.5)	115.5	6.78 (d, 8.8)
4b	159.3	-	158.2	-	157.7	-
7b	91.0	5.96 (d, 9.9)	90.0	5.95 (d, 9.7)	89.5	5.96 (d, 10.0)
8b	53.9	4.71 (d, 9.9)	52.8	4.71 (d, 9.7)	52.3	4.71 (d, 10.0)
9b	140.7	-	139.7	-	138.2	-
10b	121.9	-	120.9	-	119.2	-
11b	160.4	-	159.3	-	161.2	-
12b	107.2	6.74 (d, 2.1)	106.2	6.72 (d, 1.8)	96.4	6.24 (d, 1.8)

13b	161.7	-	160.6	-	160.3	-
14b	97.6	6.25 (d, 2.1)	96.6	6.25 (d, 1.8)	105.3	6.6 (d, 1.8)
1c	133.5	-	132.5	-	131.5	-
2c/6c	129.7	7.06 (d, 8.4)	128.6	7.08 (d, 8.5)	128.1	7.06 (d, 8.3)
3c/5c	117.1	6.80 (d, 8.7)	116.1	6.79 (d, 8.5)	115.5	6.79 (d, 8.3)
4c	159.4	-	158.3	-	157.8	-
7c	96.6	4.92 (d, 6.3)	95.6	4.90 (d, 6.4)	95.1	4.91 (d, 6.3)
8c	56.7	4.63 (d, 6.3)	55.6	4.61 (d, 6.4)	55.2	4.62 (d, 6.3)
9c	139.7	-	138.7	-	140.7	-
10c	120.7	-	119.7	-	118.3	-
11c	161.9	-	160.8	-	161.1	-
12c	106.8	6.61 (d, 1.8)	105.8	6.59 (d, 1.8)	97.5	6.23 (d, 1.8)
13c	162.8	-	161.7	-	160.2	-
14c	97.9	6.27 (d, 1.8)	96.9	6.22 (d, 1.8)	108.0	6.00 (d, 1.8)

** (Tanaka, Ito, Nakaya, Iinuma, & Riswan, 2000) [Measure acetone-d_6, 400 MHz (^{1}H) and 100 MHz (^{13}C)]

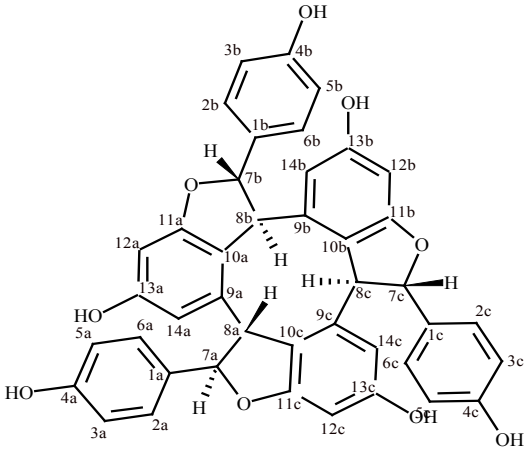

Figure 1 α-viniferin

Compound (2) resveratrol (Figure 2) was successfully isolated as white amorphous powder. ^{13}C NMR (75 MHz, acetone-d_6) displayed ten carbon signals representing a monomer stilbene (14 carbons). The typical of monomer skeleton was supported by the presence of two oxyaryl carbon signals at δ_C 157.4 (C-4'), 158.8 (C-3/5) and two aromatic quartenary carbons at δ_C 139.9 (C-1) and 129.0 (C-1'). The oxygenated carbons at δ_C 158.8 and 157.4 correlated to the hydroxyl group at δ_H 8.39 and 8.12. Nine aromatic methine carbon signals at δ_C 128.2 (C-8), 127.8 (C-2'/6'), 125.9 (C-7), 115.5 (C-3'/5'), 104.7 (C-2/6) and 101.8 (C-4) corresponded to the resveratrol skeleton. The trans olefinic C-8 and C-7 allied to the trans-coupled proton at δ_H 6.96 (1H, d, J = 18 Hz, H-8) /6.82 (1H, d, J = 18 Hz, H-7). ^{1}H NMR (300 MHz, in Acetone-d_6) exposed two sets of *ortho*-coupled aromatic protons on a *p*-substituted phenyl moieties at δ_H 7.27 (2H, d, J = 9 Hz, H-2', 6') and δ_H 6.78 (2H, d, J = 9 Hz, H-3', 5'). The spectrum also presented one set of *meta*-coupled aromatic proton δ_H 6.48 (2H, d, J = 3 Hz, H-2, 6) and one *meta*-triplet at δ_H 6.21 (1H, t, J = 3 Hz, H-4) on a 1,3,5-trisubstituted benzene ring. A pair of trans-coupled olefinic proton at δ_H 6.96 (1H, d, J = 18 Hz, H-8) /6.82 (1H, d, J = 18 Hz, H-7) were also detected. Three phenolic hydroxyl group [δ_H at 8.39 and 8.12 (1H each, s] indicated that **compound 2** was a monomer stilbene skeleton with comparison with previous data (Chu et al., 2005; Commodari, et al., 2005). The detailed of ^{1}H and ^{13}C NMR are listed in **Table 2**.

Table 2 The NMR spectral data of Compound 2 in acetone-d at 300 MHz (^{1}H) and 75 MHz (^{13}C)

No	Compound 2		resveratrol *		resveratrol**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	139.9	-	139.2	-	139.2	-
2/6	104.7	6.48 (d, 3)	104.3	6.37 (d)	104.2	6.34 (d, 2.2)
3/5	158.8	-	158.5	-	158.3	-
4	101.8	6.21 (t, 3)	101.7	6.11 (s)	101.7	6.07 BRS. 2.2
7	125.9	6.82 (d, 18)	125.6	6.78 (d, 16.4)	125.6	6.76 (d, 16)
8	128.2	6.96 (d, 18)	127.8	6.90 (d, 16.4)	128.0	6.87 (d,16)
1’	129.0	-	128.0	-	128.1	-
2’/6’	127.9	7.37 (d, 9)	127.8	7.39 (d)	127.8	7.35 (d, 8.8)
3’/5’	115.5	6.78 (d, 9)	115.5	6.73 (d)	115.4	6.71 (d, 8.8)
4’	157.4	-	157.2	-	157.2	-

* (Chu et al., 2005) ^{1}H NMR (400MHz, DMSO)
** (Adams, Ahn, Ainuddin, & Lee, 2011) (solvent DMSO)

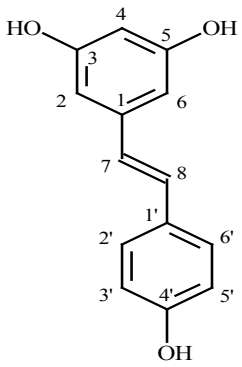

Figure 2. Resveratrol

DPPH free radical scavenging activity

The scavenging of α-viniferin was evaluated by DPPH assay method as with slight modification (Adams et al., 2011). The free radical DPPH with an odd electron gives a maximum aabsorption at 517 nm, however their absorption will decrease if reacted with antioxidants, as the DPPH becomes paired off in the presence of a hydrogen donor. In this study, the compound did not show the scavenging activity greater than 50%. **Table 3** shows the percent DPPH inhibition for α-viniferin.

Table 3. DPPH radical scavenging inhibitions α-viniferin

Concentration (µg/ml)	DPPH scavenging activitiy (%)
5	3.96
10	7.94
20	12.70
40	14.29
60	17.46
80	37.14
100	41.90

Strong radical-scavenging activity: $IC_{50} \leq 30$ µg/ml.

Moderate radical-scavenging activity: 30 µg/ml $IC_{50} \leq 100$ µg/ml.
Weak radical-scavenging activity: $IC_{50} > 100$ µg/ml.

In this study, α-viniferin did not show the scavenging activity greater than 50%. The trimer oligostilbenoid, which contain three benzofuran rings in its skeleton displayed activity 41.90 % at 100 µg/ml. This fact suggested that the number of benzofuran ring in the oligostilbenoids did not contribute significantly to the scavenging activity. It presented that α-viniferin are weak radical-scavenging activity where $IC_{50} > 100$ µg/ml.

Conclusion

Resveratrol and α-viniferin were the monomer and trimer stilbene that were successfully isolated from the stem bark of *S. ovalis*. Resveratrol was reported for the first time from this plant while α-viniferin was reported for the first time for their antioxidant activity. It indicated that α-viniferin denoted weak-radical scavenging activity.

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Conflict of interests

The authors declare that there is no conflict of interest.

References

Adams, J. M., Ahn, S., Ainuddin, N., & Lee, M.-L. (2011). A further test of a palaeoecological thermometer: Tropical rainforests have more herbivore damage diversity than temperate forests. *Review of Palaeobotany and Palynology*, *164*(1–2), 60–66. http://doi.org/10.1016/j.revpalbo.2010.11.005

Appanah, S., & Turnbull, J. M. (1998). *A Review of Dipterocarps: Taxonomy, ecology and silviculture*. *Journal of Biogeography*. http://doi.org/10.2307/2844700

Ashton, P. S. (1982). Dipterocarpaceae. *Book Flora Malesiana*, *9*(2), 237–552. Retrieved from http://www.cabdirect.org/abstracts/19820681886.html;jsessionid=F5945E7F8038E4B87EA2764A52A8F622

Cao, C.-P. (2006). *Genetic variation of the genus Shorea (Dipterocarpaceae) in Indonesia*. Georg-August University of Gottingen.

Chu, X., Sun, A., & Liu, R. (2005). Preparative isolation and purification of five compounds from the Chinese medicinal herb Polygonum cuspidatum Sieb. et Zucc by high-speed counter-current chromatography. *Journal of Chromatography A*, *1097*(1–2), 33–39.

http://doi.org/10.1016/j.chroma.2005.08.008

Commodari, F., Khiat, A., Ibrahimi, S., Brizius, A. R., & Kalkstein, N. (2005). Comparison of the phytoestrogentrans-resveratrol (3,4′,5-trihydroxystilbene) structures from x-ray diffraction and solution NMR. *Magnetic Resonance in Chemistry*, *43*(7), 567–572. http://doi.org/10.1002/mrc.1583

Noviany. (2009). The Isolation of α -viniferin , A Trimer Stilbene , from Shorea ovalis Blume. *Modern Applied Science*, *3*(4), 45–51.

Rohaiza, S. (2011). Cytotoxic oligostilbenes from Shorea hopeifolia. *African Journal of Pharmacy and Pharmacology*, *5*(10), 1272–1277. http://doi.org/10.5897/AJPP10.386

Tanaka, T., Ito, T., Iinuma, M., Ohyama, M., Ichise, M., & Tateishi, Y. (2000). Stilbene oligomers in roots of Sophora davidii. *Phytochemistry*, *53*(8), 1009–1014. http://doi.org/10.1016/S0031-9422(00)00016-9

Tanaka, T., Ito, T., Nakaya, K., Iinuma, M., & Riswan, S. (2000). Oligostilbenoids in stem bark of Vatica rassak. *Phytochemistry*, *54*(1), 63–69. http://doi.org/10.1016/S0031-9422(00)00026-1

Wong, T. M. (2002). *A-Dictionary - of -Malaysian-Timber*. (S. C. Lim & R. C. . Chung, Eds.) (2nd ed.). Perpustakaan Negara Malaysia.