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Chemical Constituents and Radical Scavenging Activities from the Twigs of *Dipterocarpus kerrii* King

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The *Dipterocarpus* genus is a member of the Dipterocarpaceae family, which exhibits a broad distribution in the tropical region of Southeast Asia, particularly in countries such as Malaysia, Cambodia, Indonesia, Thailand, and the Philippines. There has been limited research conducted thus far regarding the chemical constituents and bioactivities of this genus. Hence, *Dipterocarpus kerrii* was selected to be phytochemically studied as well as to evaluate the radical scavenging properties of the crude extract. The twigs of *D. kerrii* were collected from Jengka Forest Reserve, Universiti Teknologi MARA, Cawangan Pahang and air-dried for two weeks before being ground to granulated form. The granules (650 g) were macerated in acetone, followed by filtration and evaporation to give a crude acetone extract. The crude acetone extract (178 g) was subjected to fractionation and purification by vacuum liquid chromatography and column chromatography respectively to afford two resveratrol oligomers namely (+)-α-viniferin **(1)** (12 mg) and (-)-laevifonol **(2)** (5 mg). In addition, the crude acetone extract that was tested against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals exhibited potent radical scavenging activities with the IC_{50} values of 11.20 ± 0.54 and 32.37 ± 0.28 µg/mL, respectively.

Keywords: *Dipterocarpus kerrii*; resveratrol oligomers; (+)-α-viniferin; (-)-laevifonol; radical scavenging

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Dipterocarpaceae is the most dominant tribe in tropical lowland rainforests consisting of several genera that possess considerable relevance to human health. This family comprises 17 genera including *Shorea*, *Anisoptera*, *Hopea* and *Dipterocarpus* which has approximately 695 species [1]. *Dipterocarpus* is the most diverse genus and the third largest in the Dipterocarpaceae family. It consists of approximately 75 species and is mainly distributed in tropical Southeast Asian countries such as Malaysia, Cambodia, Indonesia, Thailand and the Philippines [2]. This genus was reported to possess plenty of potential biological activities such as antioxidant, antibacterial, anti-inflammatory [3-5] due to the presence of their resveratrol oligomers, sesquiterpenes, terpenoids and flavonoids [6-9].

The previous studies on the resin of *Dipterocarpus kerrii* in 1959 and 1991 have reported the isolation of myricetin, quercetin, procyanidin, prodelphindin, [10] α-gurjunene and ϒ-gurjunenol [11]. To date, phytochemical study on *D. kerrii* has not been thoroughly investigated. Thus, this study is conducted to isolate the resveratrol oligomers and evaluate the radical scavenging activity of the crude acetone extract of *D. kerrii*.

EXPERIMENTAL

Sample Collection

The twigs of *D. kerrii* were collected from Jengka Forest Reserve, Universiti Teknologi MARA, Cawangan Pahang in April 2022. The species was identified by Siti Munirah Mat Yunoh, a botanist from Forest Research Institute Malaysia (FRIM) and the voucher specimen (FSG5) was deposited at FRIM herbarium. The twigs were cut into small pieces and air-dried for two weeks. The dried sample was ground into granulated form.

Sample Extraction

The granules (650 g) were macerated in 5 L acetone at 25 °C for 24 hours and repeated three times. Following filtration through 125 mm-diameter Whatman No. 1 filter paper, the extract was concentrated using a rotary evaporator with a rotation speed of 100 rpm at the

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temperature of 50 °C to obtain crude acetone extract.

Chromatographic Method

The silica gel of Merck Kiesel gel 60 F_{254} was employed for thin layer chromatography (TLC) analysis on an aluminium plate. Using a fine glass capillary tube, TLC plates are spotted and then submerged in a chromatographic chamber that has been loaded with a solvent mixture of *n*-hexane:ethyl acetate. The spots were visualised under 254 nm and 356 nm UV light. Silica gel 60 (MERCK 1.07747) was used in vacuum liquid chromatography (VLC) while the silica gel of 70-230 mesh was utilised for sample preparation. As for column chromatography (CC), silica gel 60 (0.2 – 0.5 mm) (MERCK 1.07733) was used to prepare the column and silica gel 60 (0.040 – 0.063 mm) (1.09385) was used to prepare the sample.

DPPH Radical Scavenging Assay

The DPPH radical scavenging assay was performed with slight modification to the procedure outlined by Chen et al. (2021) [12]. An amount of 0.1 mM of DPPH reagent was prepared by mixing 4 mg of DPPH powder in 100 mL of methanol (MeOH). 100 µL of 5 mg/mL of crude extract and 100 µL of DPPH solution were added to a 96-well plate (well A1). For wells A2 until A11, 100 µL crude extract with different concentrations using two-fold serial dilution with MeOH was added with 100 µL DPPH solution. As a control, 100 µL of MeOH was added to A12 and mixed with 100 µL of DPPH solution. The mixture was incubated at 25 °C for 30 minutes in a dark room. The absorbance was measured at 517 nm wavelength. The DPPH radical scavenging activity of the crude extract was calculated using the following formula:

$$\text{Percentage Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100\%$$

The IC_{50} value was calculated to determine the 50% inhibition of DPPH scavenging activity using a prism. Ascorbic acid was used as a positive control.

Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging assay was carried out in accordance with Luqman and Kumar's protocol [13]. A reaction mixture was prepared by dissolving 3.75 mM deoxyribose (50mg), 1 mM hydrogen peroxide, H_2O_2 (10 µL), 100 µM iron (III) chloride, $FeCl_3$ (1.6 mg) and 100 µM ascorbic acid (1.761 mg) in 100 mL phosphate buffer. 1% thiobarbituric acid (TBA) (500 mg of TBA powder dissolved in 50 mL of sodium hydroxide, NaOH) and 2% trichloroacetic acid (TCA) (1 g of TCA powder dissolved in 50 mL distilled water) were prepared. In a 96-well plate, 20 µL of 5 mg/mL crude extract was added to well A1 with 150 µL reaction mixture. Then, for wells A2 until A11, 20 µL of crude extract with different concentrations using serial dilution with phosphate buffer was added with 150 µL reaction mixture. The mixture was then incubated at 37 °C for 60 minutes followed by cooling at room temperature. Next, 75 µL each of 2% TCA and 1% TBA were added and the reaction mixture was heated at 100 °C for 15 minutes. The absorbance was measured at 532 nm wavelength. The hydroxyl scavenging activity of various concentrations was calculated by the following equation:

$$\text{Percentage Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100\%$$

The IC_{50} value was calculated to determine the 50% inhibition of hydroxyl scavenging activity using a prism. Ascorbic acid was used as a positive control.

Isolation and Purification

The crude acetone extract (178 g) was subjected to a 20 cm diameter vacuum liquid chromatography (VLC) with the solvent system *n*-hexane:ethyl acetate in increasing polarity. The volume of each collection was 100 mL which was further concentrated using a rotary evaporator to afford four fractions, DK1- 4. Fraction DK3 (992 mg) was subjected to column chromatography (CC) using the eluent $CHCl_3$:MeOH with the ratio 9:1 in ascending polarity afforded (+)-α-viniferin (**1**) (12 mg). Sub-fraction DK3_47 (221 mg) was re-fractionated by column chromatography (CC) with the eluent $CHCl_3$:MeOH with the ratio 8:2 in increasing polarity to yield laevifonol (**2**) (5 mg).

RESULTS AND DISCUSSION

Isolated Compounds from *Dipterocarpus kerrii*

Compound **1** (12 mg) (**Figure 1**) was obtained as a brownish semisolid. The ^{1}H NMR spectrum displayed six *ortho*-coupled doublet signals at δ_H 7.20 (*J* = 8.4 Hz, H-2b,6b), 7.03 (*J* = 8.4 Hz, H-2c,6c), 7.01 (*J* = 8.4 Hz, H-2a,6a), 6.78 (*J* = 8.4 Hz, H-3c,5c), 6.76 (*J* = 8.4 Hz, H-3b,5b) and 6.71 (*J* = 8.4 Hz, H-3a,5a) representing three units of 1,4-disubstituted benzene rings (A_1, B_1 and C_1). The *meta*-coupled doublet signals at δ_H 6.72 (*J* = 1.8 Hz, H-12b), 6.59 (*J* = 1.8 Hz, H-12c), 6.24 (*J* = 1.8 Hz, H-14b), 6.23 (*J* = 1.8 Hz, H-14c) and 6.21 (*J* = 1.8 Hz, H-14a) indicated the presence of three units of 1,2,3,5-tetrasubstituted benzene rings (A_2, B_2 and C_2).

Six methine aliphatic signals were observed at δ_H 6.06 (s, H-7a), 5.93 (d, *J* = 10.0 Hz, H-7b), 4.90 (d, *J* = 6.6 Hz, H-7c), 4.69 (d, *J* = 10.0 Hz, H-8b), 4.60 (d, *J* = 6.6 Hz, H-8c) and 3.96 (s, H-8a) which suggest the presence of three 1,2-dihydrobenzofuran rings in this structure. The occurrence of singlet signals at H-7a and H-8a, but doublet signals at H-7b and H-8b, could be explained by the quick interchange of chemical reactions that allow it to appear as a singlet [14]. The ^{1}H-^{1}H COSY (**Figure 2**) experiment showed the

correlations between all methine protons (δ_H 4.69, 4.60 and 3.96) with their respective oxymethine protons (δ_H 5.93, 4.90, and 6.06) which further supported the presence of three furan rings. The correlations between the six *ortho*-coupled signals at δ_H 7.20 - 6.71 were also observed.

According to Kitanaka and co-researchers, the methine protons of H-7a and H-8a exhibited a torsion angle of 90° while the remaining pairs (H-7c and H-8c, H-7b and H-8b) are vicinally coupled methine protons. The coupling constant values of H-7b/8b and H-7c/8c are also in accordance with the values in the literature which concluded that both H-7b/8b and H-7c/8c are in *trans*-configuration [15].

The ^{13}C-APT NMR spectrum showed nine oxyaryl carbons at δ_C 161.8 (C-13c), 161.7 (C-13a), 160.9 (C-11c), 160.7 (C-13b), 159.4 (C-11a, 11b), 158.4 (C-4c), 158.3 (C-4b), 157.9 (C-4a) which support the presence of three resveratrol units (A_1, A_2, B_1, B_2, C_1 and C_2). Nine quaternary aromatic carbons were observed at δ_C 141.3 – 118.8 while the signals at δ_C 128.7 – 96.6 showed the presence of methine aromatic carbons. The remaining signals that belong to methine aliphatic carbons indicated the occurrence of three units of 1,2-dihydrobenzofuran ring that led to the formation of a cyclononane ring. Based on the comparison with the literature data by Kitanaka et al. 1990 [15], compound **1** is elucidated as a trimer resveratrol, (+)-α-viniferin (**Table 1**).

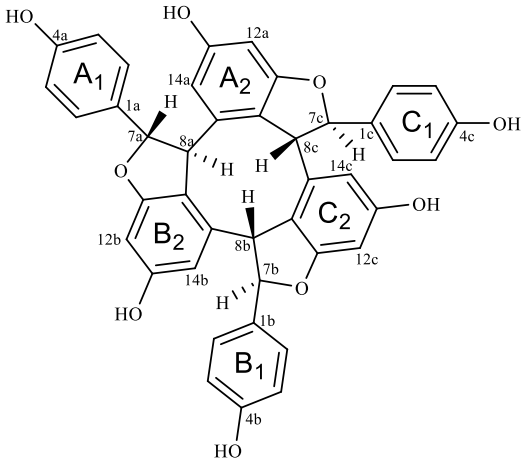

Figure 1. Structure of (+)-α-viniferin (**1**).

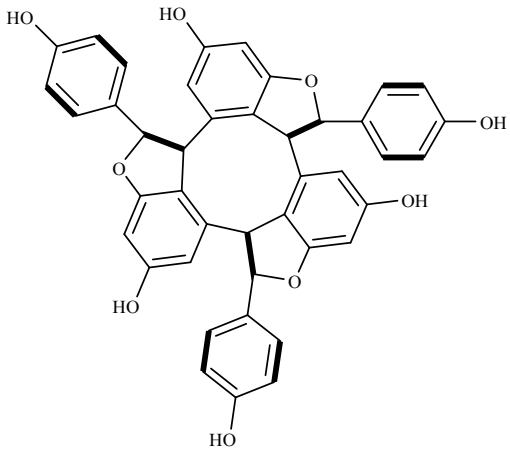

Figure 2. Selected ^{1}H-^{1}H COSY (▬) correlations of **1**.

Table 1. The comparison of ^{1}H and ^{13}C-APT NMR spectral data of compound **1**[a] and literature data (**1**[b]) by Kitanaka et al. 1990 [15].

Position	^{1}H (multiplicity, J in Hz)		δ_C	
	1[a]	**1**[b]	**1**[a]	**1**[b]
1a	-	-	132.1	132.0
2a,6a	7.01 (d, 8.4, 2H)	7.03 (d, 8.5, 2H)	128.1	128.0
3a,5a	6.71 (d, 8.4, 2H)	6.72 (d, 8.5, 2H)	115.7	115.7
4a	-	-	157.9	157.8
7a	6.06 (s, 1H)	6.07 (s, 1H)	86.4	86.4
8a	3.96 (s, 1H)	3.97 (s, 1H)	46.4	46.4
9a	-	-	141.3	141.2
10a	-	-	118.8	118.8
11a	-	-	159.4	159.3
12a	5.99 (d, 1.8, 1H)	5.99 (d, 1.8, 1H)	108.6	108.5
13a	-	-	161.7	161.6
14a	6.21 (d, 1.8, 1H)	6.22 (d, 1.8, 1H)	98.0	98.0
1b	-	-	132.4	132.3
2b,6b	7.20 (d, 8.4, 2H)	7.22 (d, 8.5, 2H)	128.2	128.1
3b,5b	6.76 (d, 8.4, 2H)	6.77 (d, 8.5, 2H)	116.1	116.1
4b	-	-	158.3	158.2
7b	5.93 (d, 10.0, 1H)	5.95 (d, 9.7, 1H)	90.0	90.0
8b	4.69 (d, 10.0, 1H)	4.71 (d, 9.7, 1H)	52.9	52.8
9b	-	-	139.8	139.7
10b	-	-	120.9	120.9
11b	-	-	159.4	159.3
12b	6.72 (d, 1.8, 1H)	6.72 (d, 1.8, 1H)	106.2	106.2
13b	-	-	160.7	160.6
14b	6.24 (d, 1.8, 1H)	6.25 (d, 1.8, 1H)	96.6	96.6
1c	-	-	132.6	132.5
2c,6c	7.03 (d, 8.4, 2H)	7.08 (d, 8.5, 2H)	128.7	128.6
3c,5c	6.78 (d, 8.4, 2H)	6.79 (d, 8.5, 2H)	116.1	116.1
4c	-	-	158.4	158.3
7c	4.90 (d, 6.6, 1H)	4.90 (d, 6.4, 1H)	95.6	95.6
8c	4.60 (d, 6.6, 1H)	4.61 (d, 6.4, 1H)	55.7	55.6
9c	-	-	138.8	138.7
10c	-	-	119.8	119.7
11c	-	-	160.9	160.8
12c	6.59 (d, 1.8, 1H)	6.59 (d, 1.8, 1H)	105.8	105.8
13c	-	-	161.8	161.7
14c	6.23 (d, 1.8, 1H)	6.22 (d, 1.8, 1H)	96.6	96.9

1[a] NMR spectra recorded at 600 MHz (^{1}H) and 150 MHz (^{13}C-APT) in acetone-d_6
1[b] NMR spectra recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C) in acetone-d_6

Compound **2** (5 mg) (**Figure 3**) was obtained as a brownish semisolid. The ^{1}H NMR spectrum displayed four *ortho*-coupled doublet signals at δ_H 6.97 (J = 9.0 Hz, H-2b,6b), 6.78 (J = 9.0 Hz, H-2a,6a) and 6.76 (J = 9.0 Hz, H-3a,5a; H-3b,5b) indicating the presence of 1,4-disubstituted benzene rings (A_1 and B_1). The presence of 1,3,5-trisubstituted benzene ring (A_2) was observed by a *meta*-coupled doublet signal at δ_H 5.92 (J = 2.0 Hz, H-10a, 14a) and a triplet signal at δ_H 6.17 (J = 2.0 Hz, H-12a). In addition, a doublet signal at δ_H 6.19 (J = 2.0 Hz, H-12b) and a broad singlet signal at δ_H 7.17 (H-14b) represents 1,2,3,5-tetrasubstituted benzene ring (B_2). The presence of rings A_1, A_2, B_1 and B_2 suggests that this compound is a resveratrol dimer. The presence of furan rings can be seen from the doublet signals at δ_H 5.30 (J = 10.8 Hz,

H-7b), 3.29 (J = 10.8 Hz, H-8b), 5.04 (J = 7.2 Hz, H-7a) as well as a broad singlet signal at δ_H 3.21 (H-8a). The coupling constant that was shown in H-7a suggested that both protons H-7a and H-8a are in *trans*-configuration [16].

There were additional signals at δ_H 4.43 (br s, H-4'), 4.24 (m, H-5'), 4.06 (dd, J = 9.6, 4.2 Hz, H-6') and 3.98 (dd, J = 9.6, 4.2 Hz, H-6") which suggest the presence of a bicyclic ascorbic acid moiety in the structure. The signal of H-4' that appeared as a broad singlet rather than a doublet may be attributed to the low resolution of the NMR [14].

The ^{1}H-^{1}H COSY (**Figure 4**) experiment exhibited all the neighbouring *ortho*-coupled signal correlations. In addition, the correlation between methine and oxymethine protons of two furan rings at δ_H 3.21 and 5.04 as well as δ_H 3.29 and 5.30 respectively, was also observed. The methine proton of ascorbic acid moiety (H-5') displayed a correlation with its neighbouring methylene protons, H-6'and H-6". Nevertheless, as seen in the structure, H-5' is bonded to a heteroatom which is oxygen from the OH group. As a result, it is either coupled weakly or not at all to its neighbouring proton, H-4'. This is caused by the rapid proton exchange with solvent or other sample molecules [17].

The ^{13}C-APT NMR spectrum demonstrated six oxyaryl carbons at δ_C 161.1 (C-11b), 159.8 (C-11a,13a), 159.0 (C-13b), 158.6 (C-4b), 158.3 (C-4a) supporting the presence of two resveratrol units (A_1, A_2, B_1 and B_2). Five quaternary aromatic signals were observed at δ_C 145.9 (C-9a), 132.4 (C-1a), 131.9 (C-1b), 129.9 (C-9b) and 122.9 (C-10b). Meanwhile, the signals at δ_C 129.1 – 97.1 belong to the methine aromatic carbons. The presence of carbon signals at δ_C 94.3 (C-7a), 90.1 (C-7b) and 56.8 (C-8a,8b) represents two furan rings. Additionally, the signal at δ_C 172.1 (C-1') together with the remaining signals at the upfield region revealed the occurrence of a bicyclic ascorbic acid moiety in compound **2**. Based on the comparison with the literature data by Hirano et al. [16], compound 2 is elucidated as a dimer resveratrol, (-)-laevifonol (**Table 2**).

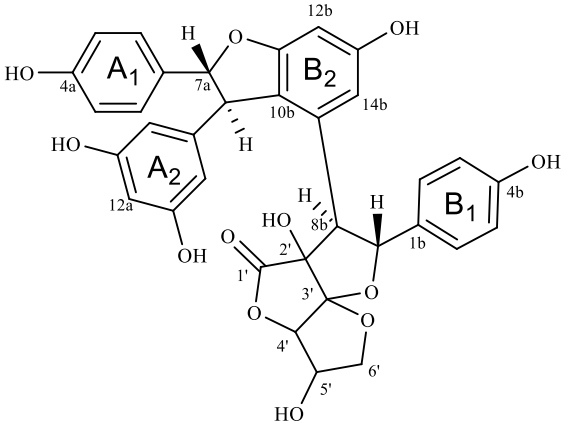

Figure 3. Structure of (-)-laevifonol (**2**).

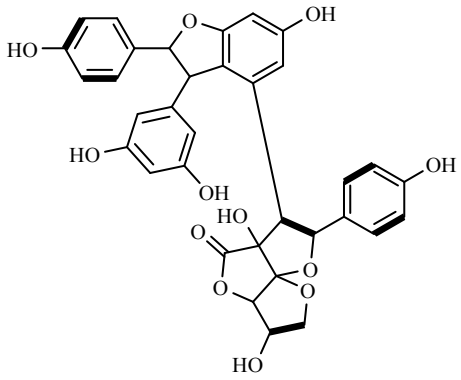

Figure 4. Selected ^{1}H-^{1}H COSY (▬) correlations of **2**.

Table 2. The comparison of ^{1}H and ^{13}C-APT NMR spectral data of compound **2**[a] and literature (**2**[b]) data by Hirano et al. 2003 [16].

Position	^{1}H (multiplicity, J)		δ_C	
	2[a]	**2**[b]	**2**[a]	**2**[b]
1a	-	-	132.4	132.4
2a,6a	6.78 (d, 9.0, 2H)	6.71 (m, 2H)	129.1	129.0
3a,5a	6.76 (d, 9.0, 2H)	6.71 (m, 2H)	115.8	115.8
4a	-	-	158.3	158.2
7a	5.04 (d, 7.2, 1H)	5.03 (d, 8.0, 1H)	94.3	94.8
8a	3.21 (br s, 1H)	3.15 (br d, 7.1, 1H)	56.8	57.1
9a	-	-	145.9	145.9
10a,14a	5.92 (d, 2.0, 2H)	5.86 (d, 2.0, 2H)	107.2	107.5
11a	-	-	159.8	159.6
12a	6.17 (t, 2.0, 1H)	6.14 (t, 2.0, 1H)	102.4	102.5
13a	-	-	159.8	159.6
1b	-	-	131.9	131.7
2b,6b	6.97 (d, 9.0, 2H)	6.92 (d, 8.5, 2H)	128.2	128.2
3b,5b	6.76 (d, 9.0, 2H)	6.71 (m, 2H)	116.1	116.1
4b	-	-	158.6	158.8
7b	5.30 (d, 10.8, 1H)	5.29 (d, 11.3, 1H)	90.1	90.8
8b	3.29 (d, 10.8, 1H)	3.22 (d, 11.0, 1H)	56.8	57.1
9b	-	-	129.9	129.8
10b	-	-	122.9	123.3
11b	-	-	161.1	161.3
12b	6.19 (d, 2.0, 1H)	6.17 (d, 2.0, 1H)	97.1	96.6
13b	-	-	159.0	159.0
14b	7.17 (br s, 1H)	7.13 (br s, 1H)	110.8	111.0
1’	-	-	172.1	173.6
2’	-	-	81.1	81.3
3’	-	-	118.7	119.1
4’	4.43 (br s, 1H)	4.36 (br s, 1H)	89.1	89.6
5’	4.24 (m, 1H)	4.20 (m, 1H)	74.7	74.6
6’	4.06 (dd, 9.6, 2.4, 1H)	4.09 (dd, 10.0, 2.0, 1H)	75.7	75.9
6”	3.98 (dd, 9.6, 4.2, 1H)	3.98 (dd, 10.3, 4.3, 1H)	75.7	75.9

2[a] NMR spectra recorded at 600 MHz (^{1}H) and 150 MHz (^{13}C-APT) in acetone-d_6
2[b] NMR spectra recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C) in methanol-d_4

Radical Scavenging Activities

The radical scavenging activities from the crude acetone extract of *D. kerrii* were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals. According to the data presented in **Table 3**, the IC_{50} values of DPPH and hydroxyl radicals were seen to be lower (11.20 ± 0.54 and 32.37 ± 0.28 µg/mL, respectively) in comparison to the standard ascorbic acid (32.36 ± 0.98 and 42.99 ± 0.48 µg/mL, respectively). These findings demonstrated that the crude extract of *D. kerrii* possesses promising anti-oxidant properties with significant IC_{50} values. The ability of *D. kerrii* crude extract to scavenge hydroxyl and DPPH radicals is thought to be contributed by the presence of polyphenols in the crude extract. In addition, a number of recent investigations have documented the DPPH radical scavenging properties exhibited by the members of the *Dipterocarpus* genus. In a prior investigation conducted by Yongram et al. (2019) [4], the crude extract of *D. alatus* demonstrated significant activity against the DPPH and ABTS radicals with the IC_{50} values of 5.76 ± 0.19 and 9.37 ± 0.03 µg/mL, respectively, in comparison to the positive control Trolox (3.93 ± 0.02 and 10.20 ± 0.10 µg/mL, respectively). Additionally, the methanol extract from *D. verrucosus and D. cornutus* showed moderate radical scavenging activity against DPPH with the IC_{50} values of 80 µg/mL and 210 µg/mL [18].

Table 3. Radical scavenging activities of crude acetone extract from *Dipterocarpus kerrii*.

Radical Scavenging Assays	**IC_{50}** (μg/mL)
DPPH	
Crude acetone extract	11.20 ± 0.54
Ascorbic acid	32.36 ± 0.98
Hydroxyl	
Crude acetone extract	32.37 ± 0.28
Ascorbic acid	42.99 ± 0.48

CONCLUSION

The phytochemical study on the twigs of *D. kerrii* has resulted in the isolation of two resveratrol oligomers elucidated as (+)-α-viniferin (**1**) and (-)-laevifonol (**2**). To the best of our knowledge, this is the first occurrence of these compounds in this species. Furthermore, the crude acetone extract from the twigs of *D. kerrii* was assessed for its ability to scavenge DPPH and hydroxyl radicals. The results revealed that the extract exhibited significant radical scavenging activity, comparable to that of ascorbic acid. Further study will be carried out on other bioactivities to assess the potential of the crude and pure compounds from this species.

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