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CHEMICAL CONSTITUENTS AND ANTI-INFLAMMATORY EFFECTS OF SOME STILBENOIDS FROM *DIPTEROCARPUS RETUSUS* FRUITS OF VIET NAM

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Abstract. Abstract. *Dipterocarpus retusus* Blume growing in Northern Viet Nam is a member of the plants family Dipterocarpaceae. Previous studies indicated that various *Dipterocarpus* species contain oleoresin, triterpenes, phenolics, stilbenoids, and coumarins with interesting biological activities. In a previous paper, we reported the isolation and characterization of five compounds, eleutherol, *trans*-resveratrol, polydatin, *β*-sitosterol, and *β*-sitosterol-3-O-*β*-D-glucopyranoside from fruits of *D. retusus* Blume. In continuation of our interest on this plant, this article describes the phytochemistry and anti-inflammatory activities of *D. retusus* Blume collected in Phu tho province. From fruits of *Dipterocarpus retusus* Blume (Dipterocarpaceae) growing in Viet Nam, five compounds, including (−)-*trans*-*ε*-viniferin (**1**), paucifloroside A (**2**), ursolic acid (**3**), quercetin (**4**), and catechin (**5**), were isolated and identified by spectroscopic methods and comparison with literature data. This study also reports the anti-inflammatory effect of methanol and ethyl acetate extracts and stilbenoid compounds via their inhibitory activity against the production of nitric oxide (NO) in RAW 264.7 macrophages cells stimulated by lipopolysaccharide (LPS). All data are presented as means of three replicates ± standard deviations. The test concentrations of 30 and 100 µg/mL all samples were capable of inhibiting NO production with varying grades of cytotoxicities. The stilbenoids: polydatin (**8**), *trans*-*ε*-viniferin (**1**) and paucifloroside A (**2**) showed both high anti-inflammatory activity and low cytotoxicity against the testing cells (IC_{50} value: polydatin = 0.46 ± 0.21 µg/mL, *trans*-*ε*-viniferin = 2.51 ± 0.35 µg/mL and paucifloroside A = 16.60 ± 1.56 µg/mL).

Keywords: Dipterocarpus retusus Blume, fruit, ε-viniferin, paucifloroside, anti-inflammatory activity.

Classification numbers: 1.1.1, 1.6.1, 1.2.1.

1. INTRODUCTION

Dipterocarpus retusus Blume is a species belonging to the family Dipterocarpaceae. *Dipterocarpus* species are distributed mainly in Southeast Asia countries such as Myanmar, Thailand, Laos, Cambodia, Malaysia, Philippines, Indonesia and Viet Nam [1]. Previous studies

indicated that various *Dipterocarpus* species contain various types of compounds, including triterpenes, phenolics, stilbenoids, and coumarins [2 - 4] and exert diverse biological activities, such as anti-inflammatory, anti-fungal, anti-bacterial, anti-oxidant and cytotoxic activities [4-7]. The bark of the *Dipterocarpus* species is often used in traditional medicine, however as far as we know, there have not been many studies on chemical composition as well as biological activity of fruit part both in Viet Nam and in the world. In a previous paper, we reported the isolation and characterization of five compounds, namely eleutherol (**6**), 3,5,4'-trihydroxy-trans-stilbene (**7**), polydatin (**8**), *β*-sitosterol (**9**), and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**10**) from fruits of *D. retusus* Blume [8]. In continuation of our interest in this plant, this article describes the phytochemical results and anti-inflammatory activities of *D. retusus* fruits collected from Phu Tho province. From the fruits of this plant, five compounds (-)-*trans*-ε-viniferin (**1**), paucifloroside A (**2**), ursolic acid (**3**), quercetin (**4**) and catechin (**5**) were isolated and characterized. This study also reports the anti-inflammatory effect of methanol andethyl acetate extracts and stilbenoid compounds via their inhibitory activity against the production of nitric oxide (NO) in RAW 264.7 macrophages cells stimulated by lipopolysaccharide (LPS).

2. MATERIAL AND METHODS

2.1. Instruments and chemicals

All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ^{1}H- and 125 MHz for ^{13}C-NMR) using TMS as an internal standard. Chemical shifts (δ) were reported in ppm. Melting point was measured on a Mikroskopheiztisch PHMK-50, VEB Waegetechnik Rapido, Germany. The FT-IR spectra were recorded on an IMPACT-410FT-IR spectrometer (CARL ZEISS JENA). Mass spectra were measured with an HRGC/MS *AutoSpec-Ultima* (England). Silica gel 60 F_{254} (Merck) was used for thin layer chromatography (TLC). Column chromatography (CC) was performed using silica gel 60 (40 - 63 μm, Merck) or YMC RP-18. Gel permeation chromatography was conducted using Sephadex LH-20 in methanol. Organic solvents were of analytical grade or redistilled.

2.2. Plant materials

The fruits of *Dipterocarpus retusus* Blume were collected in June 2018 in Phu Tho province, Viet Nam. The plants were identified by Mrs. Nguyen Kim Dao (Institute of Ecology and Biological Resources, VAST). A voucher specimen DR 16.2 has been deposited at the Institute of Chemistry (VAST), Viet Nam.

2.3. Extraction and isolation

The dried and powdered fruits of *D. retusus* Blume (1 kg) were extracted three times with methanol by sonication at 45 - 50 ^{o}C. The extracts were combined and concentrated under vacuum at 55 ^{o}C to yield 225.0 g of a crude residue, which was then suspended in water and successively partitioned with *n*- hexane, ethyl acetate and *n*-butanol (2.0 L each) to obtain the *n*-hexane (10 g), EtOAc (75 g), and *n*-BuOH (66 g) extract residues after the solvent removal *in vacuo*.

The ethyl acetate fraction (70 g) was subjected to vacuum liquid chromatography (VLC) over 90 g of silica gel (0.04 - 0.063 μm) eluting with gradient solvent systems of *n*-hexane/EtOAc (0 - 100 %) and EtOAc/MeOH (0 - 20 %) to yield 10 fractions (F1-F10). The fraction F3 (540 mg) was subjected to silica gel CC with gradient CH_2Cl_2/MeOH (1/99-8/2) as the eluent to obtain six sub-fractions (F3.1-F3.6). Fraction F3.3 gave 20.8 mg of a white powder, which was recrystallized in CH_2Cl_2/MeOH to afford compound **3** (16 mg) in the form of white crystals. Compound **4** (22 mg) was obtaind by CC of fraction 4 (206 mg) on a Sephadex LH-20 column using MeOH as the eluent. Fraction F6 (735 mg) was chromatographed on an YMC RP-18 column and eluted with water/methanol (20 - 100 %) to afford five sub-fractions (F6.1- F6.5). Compound **1** (28.6 mg) was obtained from fraction F6.5. Fraction F8 (1050 mg) was chromatographed on fast silicagel CC and eluted with CH_2Cl_2/MeOH (gradient 5 - 30 %) to yield five sub-fractions (F8.1-F8.5). Fraction F8.3 was further purified using Sephadex LH-20 column, eluted with CH_2Cl_2:MeOH (1:5) to give compound **5** (14 mg). Fraction F8.4 was purified by CC on an YMC RP-18 column, eluted with H_2O/MeOH (20 - 50 %), yielding compound **2** (35 mg).

Compound 1: (−)-*trans*-ε-viniferin

Pale brown powder; $C_{28}H_{22}O_6$, mp. 151 - 152 ^oC, $[\alpha]^{24}_D$ = −45 (*c* = 0.5, MeOH); IR_{KBr} (cm^{-1}): 3430, 2917, 2841, 1615, 1515, 1446, 1241, 1170 and 831. ESI-MS (*m/z*): 453.1 $[M - H]^-$. ^1H NMR [500 MHz, CD_3OD] δ (ppm): 7.17 (2H, d, *J* = 8.5 Hz, H-2/ 6), 6.80 (2H, d, *J* = 8.5 Hz, H-3/ 5), 5.39 (1H, d, *J* = 6.5 Hz, H-7), 4.38 (1H, d, *J* = 6.5 Hz, H-8), 6.19 (2H, d, *J* = 2 Hz, H-10, 14), 6.22 (1H, d, *J* = 2 Hz, H-12), 7.07 (2H, d, *J* = 8.5 Hz, H-2′/ 6′), 6.68 (2H, d, *J* = 8.5 Hz, H-3′/ 5′), 6.85 (1H, d, *J* = 16.5 Hz, H-7′), 6.61 (1H, d, *J* = 16.5 Hz, H-8′), 6.28 (1H, d, *J* = 2.0 Hz, H-12′), 6.66 (1H, d, *J* = 2.0 Hz, H-14′).

^{13}C NMR [125 MHz, CD_3OD] δ (ppm): 133.9 (C-1), 128.1 (C-2/ 6), 116.3 (C-3/5), 158.4 (C-4), 94.8 (C-7), 58.2 (C-8), 147.3 (C-9), 107.5 (C-10, 14), 160.0 (C-11), 102.2 (C-12), 160.0 (C-13), 130.6 (C-1′), 128.7 (C-2′/6′), 116.3 (C-3′/ 5′), 158.3 (C-4′), 130.4 (C-7′), 123.7 (C-8′), 136.9 (C-9′), 120.0 (C-10′), 162.7 (C-11′), 96.8 (C-12′), 159.7 (C-13′), 104.5 (C-14′).

Compound 2:**paucifloroside A**

Brown amorphous powder; $C_{34}H_{32}O_{11}$, $[\alpha]^{23}_D$ = –75 (*c* =0.1, MeOH); ESI-MS (*m/z*): 615.1 $[M - H]^-$.

^1H NMR (500 MHz, CD_3OD), δ (ppm): 7.17 (2H, d, *J* = 8.5 Hz, H-2/ 6), 6.80 (2H, d, *J* = 8.5 Hz, H-3/ 5), 5.40 (1H, d, *J* = 6.0 Hz, H-7), 4.46 (1H, d, *J* = 6.0 Hz, H-8), 6.29 (1H, d, *J* = 2 Hz, H-10, 14), 6.36 (1H, d, *J* = 2 Hz, H-12), 7.01 (2H, d, *J* = 8.5 Hz, H-2′/ 6′), 6.70 (2H, d, *J* = 8.5 Hz, H-3′/ 5′), 6.85 (1H, d, *J* = 16.5 Hz, H-7′), 6.59 (1H, d, *J* = 16.5 Hz, H-8′), 6.45 (1H, d, *J* = 2.0 Hz, H-12′), 6.65 (1H, d, *J* = 2.0 Hz, H-14′), 4.87 (1H, d, *J* = 8 Hz, H-glc-1), 3.44 (1H, m, H-glc-2), 3.42 (1H, m, H-glc-3), 3.73 (1H, m, H-glc-4), 3.46 (1H, m, H-glc-5), 3.80 (1H, dd, *J* = 12.0, 2Hz, H-glc-6).

^{13}C NMR (125 MHz, CD_3OD), δ (ppm): 133.9 (C-1), 128.1 (C-2/ 6), 116.3 (C-3/ 5), 158.4 (C-4), 94.7 (C-7), 58.0 (C-8), 147.6 (C-9), 108.5 (C-10, 14), 160.6 (C-11), 159.9 (C-13), 102.2 (C-12), 130.3 (C-1′), 128.8 (C-2′/ 6′), 116.4 (C-3′/ 5′), 158.5 (C-4′), 130.5 (C-7′), 123.6 (C-8′),

136.9 (C-9′), 119.9 (C-10′), 162.7 (C-11′), 96.9 (C-12′), 159.8 (C-13′), 104.5 (C-14′), 103.6 (C-glc-1), 74.7 (C-glc-2), 71.0 (C-glc-3), 77.8 (C-glc-4), 77.9 (C-glc-5), 62.1 (C-glc-6).

Compound 3:**ursolic acid**

White powder, $C_{30}H_{48}O_3$, mp. 289 - 291 °C. IR_{KBr} (cm^{-1}): 3442, 2390,1692, 1510, 1262, 1052, 996, 662. ESI-MS (*m/z*):457 $[M + H]^+$.

^{1}H-NMR ($CDCl_3$ & CD_3OD, 500 MHz), δ (ppm): 5.18 (1H, t, *J* = 3.5 Hz, H-12), 3.09 (1H, dd, *J* = 11.5, 4.5 Hz, H-3), 2.08 (1H, d, *J* = 11.5 Hz, H-18), 1.87 (3H, s, H-27), 0.96 (3H, s, H-23), 0.86 (3H, d, *J* = 6.5 Hz, H-30), 0.82 (3H, s, H-25), 0.74 (3H, d, *J* = 6.25 Hz, H-29), 0.69 (3H, s, H-26), 0.65 (3H, s, H-24).

^{13}C-NMR ($CDCl_3$ &CD_3OD, 125 MHz) δ (ppm): 38.5 (C-1), 26.6 (C-2), 78.6 (C-3), 38.7 (C-4), 55.1 (C-5), 18.1 (C-6), 32.8 (C-7), 39.3 (C-8), 47.6 (C-9), 36.7 (C-10), 23.3 (C-11), 125.3 (C-12), 138.0 (C-13), 41.8 (C-14), 27.8 (C-15), 24.0 (C-16), 47.6 (C-17), 52.6 (C-18), 39.3 (C-19), 39.0 (C-20), 30.4 (C-21), 36.7 (C-22), 27.8 (C-23), 15.1 (C-24), 15.3 (C-25), 16.6 (C-26), 23.1 (C-27), 180.4 (C-28), 16.7 (C-29), 20.8 (C-30).

Compound 4:**quercetin**

Yellow powder, $C_{15}H_{11}O_7$, mp. 314 - 315 °C, IR_{KBr} (cm^{-1}): 3385, 2948, 2830, 1672, 1518, 1455, 1026, 827. ESI-MS *m/z* 301 $[M-H]^-$.

^{1}H-NMR (CD_3OD, 500MHz), δ (ppm): 7.66 (dd, *J* = 8.5 Hz, H-6′), 7.75 (d, *J* = 2.0 Hz, H-2′), 6.91 (d, *J* = 8.5 Hz, H-5′), 6.41 (d, *J* = 2 Hz, H-8), 6.21 (d, *J* = 2 Hz, H-6).

^{13}C-NMR (CD_3OD, 125 MHz), δ (ppm): 148.7 (C-2), 137.2 (C-3), 177.3 (C-4), 162.5 (C-5), 99.

2 (C-6), 165.6 (C-7), 94.5 (C-8), 158.3 (C-9), 104.6 (C-10), 124.2 (C-1′), 116.1 (C-2′), 146.2 (C-3′), 148.0 (C-4′), 116.2 (C-5′), 121.8 (C-6′).

Compound 5:**(+)-catechin**

Light brown solid, $C_{15}H_{14}O_6$, mp. 174 - 175 °C. IR_{KBr} (cm^{-1}): 3820, 3391, 2933, 2892, 1627, 1522, 1471, 1289, 1245, 1198 and 868. ESI-MS *m/z* 289 $[M-H]^-$.

^{1}H-NMR ($CDCl_3$ & CD_3OD, 500 MHz); δ (ppm): 6.65 (dd, *J* = 8.0 Hz, 2.0 Hz, H-6′), 6.75 (d, *J* = 2 Hz , H-2′), 6.69 (d, *J* = 8.0 Hz, H-5′), 5.85 (d, *J* = 1.5 Hz, H-8), 5.81 (d, *J* =1.5 Hz, H-6), 2.8 (dd, *J* = 16 Hz, 5.5 Hz, H-4^b), 2,4 (dd, *J* = 16 Hz, 8.5 Hz, H-4^a), 3,9 (ddd, *J* = 8.8 & 5.5 Hz, H-3), 4.5 (d, *J* = 7.5 Hz, H-2).

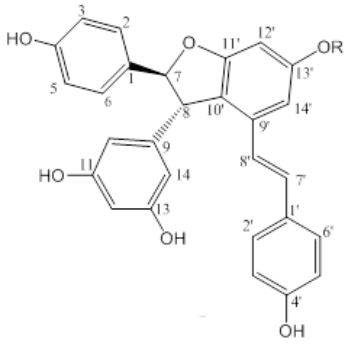
^{13}C-NMR ($CDCl_3$ & CD_3OD, 125 MHz), δ (ppm): 81.3 (C-2), 67.5 (C-3), 27.3 (C-4), 155.7 (C-5), 95.4 (C-6), 155.2 (C-7), 94.5 (C-8), 155.8 (C-9), 99.7 (C-10), 130.2 (C-1′), 114.0 (C-2′), 144.4 (C-3′), 144.6 (C-4′), 114.9 (C-5′), 119.0 (C-6′).

2.4. Anti-inflammatory activity

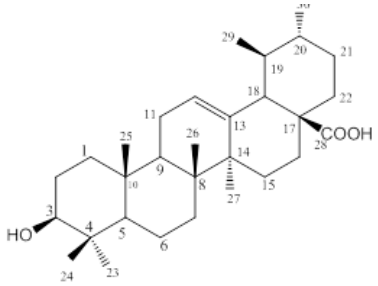
The anti-inflammatory activity of the extracts and isolated compounds was determined through the inhibition of NO production in lipopolysaccharide (LPS)-induced RAW264.7 cells (ATCC, Manassas, VA, USA) according to procedures previously described [9]. All data are presented as means of three replicates ± standard deviations. Briefly, RAW 264.7 cells (1×10^5 cells/ml) were pretreated with various sample concentrations for 30 min and then stimulated for

24 h with or without 1 µg/ml LPS at 37 °C, 5 % CO_2. Cardamonin (Sigma-Aldrich, > 98 % HPLC) was used as a positive control. The NO concentration in the culture supernatants was measured using Griess reagents (Merck KgaA, Darmstadt, Germany). Subsequently, absorbance of the mixture solution at 570 nm was measured. A standard curve was prepared using $NaNO_2$ as a standard solution in the same manner, and was used to calculate the concentration of NO. Cell viability was assayed by using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide at 0.5 mg/mL in PBS].

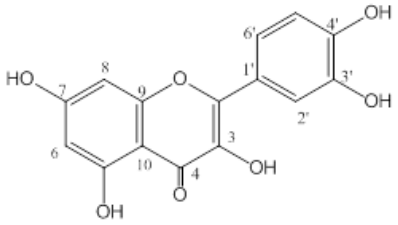
3. RESULTS AND DISCUSSION



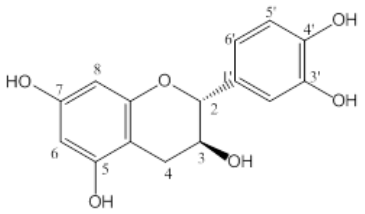
1. (-)-trans-ε-viniferin, R=H
2. paucifloroside A, R=Glu



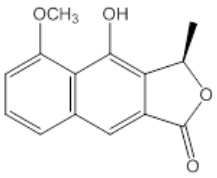
3. ursolic acid



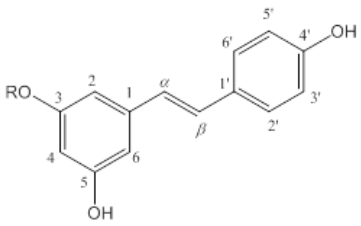
4. quercetin



5. catechin



6. eleuthrol



7. 3,5,4'-trihydroxy-trans-stilbene, R=H
8. polydatin, R= β-D-glucopyranosyl

The phytochemical investigation of the ethyl acetate extract of the dried *D. retusus* fruits resulted in the isolation of five compounds, including (-)-*trans*-ε-viniferin (**1**), paucifloroside A (**2**), ursolic acid (**3**), quercetin (**4**) and catechin (**5**). The structures of compound **1 - 5** were

elucidated by spectroscopic methods and by comparing their physical and spectroscopic data with those reported in the literature.

Compound **1** was isolated as a brown, amorphous powder. The ^{1}H NMR spectrum of **1** showed the proton signals of a 4-hydroxystyryl unit, including doublet signals at δ 7.07 (2H, d, *J* = 8.5 Hz, H-2′/6′) and 6.68 (2H, d, *J* = 8.5 Hz, H-3′/5′), together with two doublet signals at 6.85 (1H, *J* = 16.5 Hz, H-7′) and 6.61 (1H, *J* = 16.5 Hz, H-8′), suggesting the presence of *trans*-stilbene skeleton in the molecule. The ^{1}H NMR spectrum of **1** also showed characteristic resonances due to a 4-hydroxyphenyl unit at δ 7.17 (2H, d, *J* = 8.5 Hz, H-2/6) and δ 6.80 (2H, d, *J* = 8.5 Hz, H-3/5), and the presence of doublet signals at δ 6.19 (2H, d, *J* = 2.0 Hz, H10/14) and δ 6.22 (d, *J* = 2.0 Hz, H-12) due to a 3,5-dihydroxyphenyl ring. The signals at δ 6.66 (1H, d, *J* = 2.0 Hz, H-14′) and 6.28 (1H, d, *J* = 2.0 Hz, H-12′) were assigned to two *meta*-coupled aromatic protons of an 1,2,3,5-tetrasubstituted benzene ring. In addition, a pair of aliphatic protons at δ 5.39 (1H, d, *J* = 6.5 Hz, H-7) and 4.38 (1H, d, *J* = 6.5 Hz, H-8) was assigned to a 2,3-dihydrobenzofuran moiety with *trans* configuration.

The ^{13}C NMR spectrum of **1** showed 22 signals representing 28 carbons which consist of six oxy-aryl carbons at δ 162.7 (C-11′), 160.0 (C-11), 160.0 (C-13), 159.7 (C-13′), 158.4 (C-4), and 158.3 (C-4′) together with five nonhydrogenated carbons at δ 120.0 (C-10′), 130.6 (C-1′), 133.9 (C-1), 136.9 (C-9′), 147.3 (C-9). In addition, there were also seven signals of methine carbons at δ 58.2 (C-8), 123.7 (C-8′), 94.8 (C-7), 130.4 (C-7′), 102.2 (C-12), 96.8 (C-12′), and 104.4 (C-14′). The remaining signals belonged to five symmetric methine carbon signals: δ 128.7 (C-2′/6′), 128.1 (C-2/6), 116.3 (C-3′/5′), 116.3 (C-3/5) and 107.5 (C-10/14). This analysis, combined with HSQC and HMBC spectra data and compared with references [10,11,12] confirmed that compound **1** is (-)-*trans*-ε-viniferin.

Compound **2** was obtained as brown amorphous powder. The ^{1}H- and ^{13}C NMR spectral data of **2** showed close similarity to those of ε-viniferin (**1**) except for the addition of a *β*-glucopyranosyl moiety. The presence of a *β*-glucopyranosyl moiety was supported by the ^{13}C-NMR spectral signals at δ 103.6, 77.9, 77.8, 74.7, 71.0 and 62.1 together with an anomeric proton at δ 4.87 (1H, d, *J* = 8.0 Hz). To confirm the position of the glucosidic linkage and the correct ^{1}H- and ^{13}C NMR spectral assignments, HSQC, HMBC and ^{1}H-^{1}H COSY spectra of **2** were recorded and analysed. Based on the spectral data and comparison with those in the literature [13], compound **2** was identified as pauciflorocide A [13].

Previously, (-)-ε-viniferin together with (-)-α-viniferin, (-)-vaticanol A, scopoletin and (-)-bergenin were isolated from the bark of *Dipterocarpus retusus* Blume [14].

Compound **3** was obtained as white powder. The ESI-MS spectrum showed a molecular ion peak at *m/z* 457 $[M+H]^{+}$. The ^{1}H-NMR spectrum of **3** showed resonances for five tertiary methyl singlet signals at δ 0.65 (3H, H-24), 0.69 (3H, H-26), 0.82 (3H, H-25), 0.96 (3H, H-23), and 1.87 (3H, H-27), two secondary methyl doublet signals at δ 0.74 (3H, d, *J* = 6.25 Hz, H-29) and 0.86 (3H, d, *J* = 6.5 Hz, H-30), an oxygenated methine signal at δ 3.07 (1H,dd, J = 11.5 Hz, 4.5 Hz, H-3), and olefinic signals at δ 5.12 (1H, t, H-12), suggesting that **3** is a 3-*β*-hydroxy-12-ursen-type triterpenoid possessing a carboxyl group. The ^{13}C NMR spectrum revealed the presence of

30 carbons which consist of seven methyl, a carboxyl, an olefinic methine, an olefinic quaternary, and an oxygenated methine carbon. Comparison of the above data with the literature [15,16] led to the identification of **3** as ursolic acid (3*β*-hydroxy-urs-12-en-28-oic acid).

Compound **4** was isolated as yellow powder. The ^{1}H-NMR of compound **4** showed two sets of signals: the signals with *meta*-coupling (J = 2.0 Hz) at δ 6.21 (H-6) and 6.41 (H-8) assigned to protons of the A ring in the flavonoid. The another set of three signals at 7.66 (dd, J = 8.5 Hz, J = 2.0 Hz, H-6′), 6.91 (d, J = 8.5 Hz, H-5′), and 7.75 (d, J = 2.0 Hz, H-2′) is due to protons of the aromatic B-ring. The ^{13}C-NMR and DEPT spectrum showed fifteen carbon signals, which consisted of nine nonhydrogenated carbons (δC 148.7, 137.2, 162.5, 165.6, 158.2, 104.5, 124.2, 146.2, 148.0), five methines (δC 99.2, 94.5, 116.1, 116.2, 121.8), and one carbonyl carbon (δ C 177.3). All the spectral data of compound **4** were consistent with the literature data of quercetin [17, 18].

Compound **5** was isolated as light brown solid. The ^{1}H-NMR spectrum indicated the presence of two *meta* aromatic protons on ring A (H-6, H-8) and two hydroxy groups at C-5, and C-7. Four protons on ring C are located at C-2, C-3, C-4 (two protons) and one hydroxy group located at C-3. Furthermore, the ABX system of three protons on the aromatic ring B at 6.65 (1H, dd, 8.0 Hz, 2Hz, H-6′), 6.75 (1H, s, H-2′) and 6.69 (1H, d, J = 8.0, 2 Hz, H-5′) and ^{13}C-NMR data showed the presence of two hydroxyl groups at C-3′ and C-4′. The above analysis together with literature data comparison [21, 22] indicated that **5** should be (+)-catechin.

The MeOH and EtOAc extracts and the three isolated stilben compounds, namely polydatin (**6**), *trans*- ε-viniferin (**1**), and paucifloroside A (**2**) were assayed for their LPS-induced NO inhibitory activity in, and their cytotoxicity on RAW 264.7 macrophages. A compound of cardamonin was used as positive control. As seen from Table 1, at the test concentrations of 30 and 100 µg/mL all samples were capable of inhibiting NO production with varying grades of cytotoxicities.

Table 1. Inhibitory effects of the extracts and isolated compounds of *D. retusus* Blume on NO production.

№	Test samples	Samples concentration	Inhibition of NO production (%)	Cell survival (%)
	(-)	-	100.0 ± 1.3	104.76 ± 0.15
	(+) (Cardamonin)	0.3 µM	45.85 ± 2.12	86.47 ± 0.21
		3.0 µM	86.93 ± 0.96	71.8 ± 0.51
	LPS	-	0.0 ± 0.9	100.0 ± 0.13
	MeOH extract	30 µg/mL	>100 0.33	8.75 ± 2.52
		100 µg/mL	>100 ± 2.16	7.96 ± 1.38
	EtOAc extract	30 µg/mL	65.64 ± 1.64	89.29 ±2.36
		100 µg/mL	>100 ± 2.04	6.5 ± 0.61
	Polydatin (**8**)	30 µg/mL	>100 ± 0.5	85.51 ± 2.55
		100 µg/mL	>100 ± 1.74	61.53 ± 2.8
	trans-ε-viniferin (**1**)	30 µg/mL	>100 ± 2.73	54.01 ± 1.57
		100 µg/mL	>100 ± 0.33	7.36 ± 1.22
	Paucifloroside A (**2**)	30 µg/mL	58.28 ± 0.99	99.56 ± 2.53
		100 µg/mL	84.05 ± 1.14	87.89 ± 0.86

Table 2. IC_{50} values for the inhibition of NO production by the samples.

№	**Test samples**	**IC_{50} value**
1	MeOH extract	0.95 ± 0.03 µg/mL
2	EtOAc extract	12.02 ± 0.82 µg/mL
3	Polydatin (**8**)	0.46 ± 0.21 µg/mL
4	*trans*-ε-viniferin (**1**)	2.51 ± 0.35 µg/mL
5	Paucifloroside A (**2**)	16.60 ± 1.56 µg/mL
	Cardamonin	2.12 ± 0.05 µM

The stilbenoids polydatin (**8**), *trans*-ε-viniferin (**1**) and paucifloroside A (**2**) showed both high anti-inflammatory activity and low cytotoxicity against the testing cells. The MeOH extract is highly active but also highly cytotoxic. The IC_{50} values for the inhibition of NO production by the samples are shown in Table 2.

Recently, ursolic acid (**3**) and quercetin (**4**) have also been reported to possess anti-inflammatory activity [9].

4. CONCLUSIONS

This is the first report on the isolation of (-)-*trans*-ε-viniferin, paucifloroside A, ursolic acid, quercetin and (+)-catechin from fruits of *Dipterocarpus retusus* Blume growing in Viet Nam. The chemical structures of the isolates were elucidated based on NMR spectroscopy and comparison with literature data. The methanol and ethyl acetate extracts of the fruits of *D. retusus* and especially their stilbenoid constituents including polydatin, ε-viniferin, paucifloroside A revealed anti-inflammatory activities, which justified the need for more detailed study on phytochemistry and bioactivity of this plant.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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