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STUDY ON CHEMICAL CONSTITUENTS AND BIOACTIVITIES OF THE FRUITS OF *DIPTEROCARPUS RETUSUS* OF VIET NAM

Ho Dac Hung*, Doan Duy Tien, Nguyen Thi Ngoan , Ba Thi Duong, Do Quoc Viet, Pham Gia Dien, Bui Kim Anh*

Institute of Chemistry, VAST, 18 Hoang Quoc Viet St. Ha Noi

*Email: hungvhh@gmail.com; anhvhh@gmail.com

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Abstract. The first study on chemical constituents and biological activities of fruits of *Dipterocarpus retusus* Blume. (Dipterocarpaceae) (Vietnamese name: Chò nâu) growing in Vietnam was reported. Column chromatography of the ethyl acetate extract led to the isolation of five compounds: eleutherol (**1**), *trans*-resveratrol (**2**), polydatin (**3**), *β*-sitosterol (**4**) and *β*-sitosterol-3-O-*β*-D-glucopyranoside (**5**). Their structures were elucidated based on the NMR spectroscopic methods and compared with literature data. The *n*-hexane, ethyl acetate and butanol extracts from the fruits of *D. retusus* Blume were evaluated for antioxidant and cytotoxic activities. The ethyl acetate extract exhibited the cytotoxic activity against KB cell lines with IC_{50} value of 34.46 μg/ml.

Keywords: Dipterocarpus retusus Blume, eleutherol, resveratrol, polydatin.

Classification numbers: 1.1.1; 1.1.6; 1.2.1; 1.3.1.

1. INTRODUCTION

Dipterocarpus retusus Blume, a member of family Dipterocarpaceae. *D. retusus* Blume, is a large timber tree, 30 - 40 metre high, up to 1 metre in diameter that grows in Northern Vietnam. It has straight body, cylindrical, and high branches. The fruits are slightly rounded, 2-3 cm in diameter, with 5 lobes, in which 3 lobes are reduced, heart shape, rounded top, and 0.7 cm long; two lobes thrive in wings, 15-20 cm in length, 2-3 cm in width, 3 clear tendrils. It is mainly distributed in Son la, Yen Bai, Ha Giang, Tuyen Quang, Bac Kan, Phu Tho, Vinh Phuc, Hoa Binh, Quang Binh, and Quang Nam provinces [1]. Studies indicated that various *Dipterocarpus* species contain chemical constituents such as triterpenes, stilbenoid and derivative coumarin compounds, etc. with different interesting biological activities [2 - 7]. In Vietnam, there is no study on chemical constituents and biological activities of fruits. This paper reports results of the isolation and determination of the chemical structure of five compounds: eleutherol (**1**), resveratrol (**2**), polydatin (**3**), *β*-sitosterol (**4**) and *β*-sitosterol-3-O-*β*-D-glucopyranoside (**5**) from the fruits of *D. retusus* Blume. Besides, the extracts of *n*-hexane, ethyl acetate and *n*-butanol of the fruits of plant were also evaluated for cytotoxic activity and antioxidant activities.

2. MATERIAL AND METHODS

2.1. Instruments and chemicals

The ^{1}H-NMR, ^{13}C-NMR and spectra were recorded on an AVANCE spectrometer AV 500 (Bruker, Germany) in the Institute of Chemistry - Vietnam Academy of Science and Technology (VAST). Melting point was measured on Mikroskopheiztisch PHMK-50, VEB Waegetechnik Rapido, Germany. The FT-IR spectra were recorded on IMPACT-410FT-IR spectrometer (CARL ZEISS JENA). Mass spectra were measured with an HRGC/MS Auto Spec-Ultina (England). Precoated plates of silica gel 60 F_{254} were used for thin-layer chromatography (TLC). Column chromatography (CC) was carried out on silica gel 60 (Merck, 40-63 μm). Gel permeation chromatography was conducted, using Sephadex LH-20 in methanol. Organic solvents were distilled before use.

2.2. Plant materials

The fresh fruits of *D. retusus* Blume were collected in Phu Tho province of Viet Nam in June 2017, and identified by Dr. Nguyen Kim Dao (Institute of Ecology and Biological Resources). A voucher specimen (BKA-16) has been deposited in the Institute of Chemistry (VAST), Viet Nam.

2.3. Extraction and isolation

The dried powdered fruits of *D. retusus* Blume (3.0 kg) were extracted with ethanol 90 % at room temperature (4 times × 24 h). The ethanol extracts were combined and concentrated under vacuum at 55 ^{o}C. The obtained residue was suspended in water and successively partitioned with *n*-hexane (4 times), ethyl acetate (4 times) and butanol (4 times). The extracts of each solvent were concentrated under reduced pressure at 40 ^{o}C to yield *n*-hexane (27 g), EtOAc (185 g) and BuOH (128 g) residues, respectively. These extracts were tested for antioxidant and cytotoxic activities against KB cell line.

The ethyl acetate residue (85 g) was subjected to vacuum liquid chromatography (VLC) over 100 g silica gel (0.04 - 0.063 mm), eluted with gradient of *n*-hexane/EtOAc (0 % - 100 %, v/v) and EtOAc/MeOH (0 % - 20 %, v/v) to yield 10 fractions (F1-F10). From fraction F1 7.8 mg of colourless needle crystals (compound **4**) was obtained as brown powder. Fraction F2 was subjected to fast column chromatography with silica gel (0.04-0.063 mm), eluted with gradient of *n*-hexane/acetone (0 % - 10 %, v/v) to give 5 fractions (F2.1 – F2.5). Fraction F2.3 gave 25.5 mg of brown amorphous powder (compound **1**). Fraction F5 was subjected to fast column chromatography with silica gel (0.04 - 0.063 mm) eluting with gradient of CH_2Cl_2/MeOH (0 % - 20 %, v/v) to give 7 fractions (F5.1 – F5.7). Fraction F5.4 gave 10.8 mg of white needle crystals (compound **2**). Fraction F6 gave 16.6 mg of white powder (compound **5**). Fraction F7 was subjected to reverse phase column chromatography with silica gel RP 18 and eluted with gradient of H_2O/MeOH (20 % - 100 %, v/v) to give 8 fractions (F7.1 – F7.8). Fraction F7.6 gave 16.0 mg of white needle crystals (compound **3**).

Compound **1** (eleutherol): Brown amorphous powder, m.p. 203 – 204 ^{o}C. $C_{14}H_{12}O_4$. ESI-MS *m/z* 245 $[M+H]^+$.

^{1}H-NMR (500 MHz, CD_3OD), (δ ppm): 1.72 (3H, d, 6.5 Hz, 3-CH_3), 4.13 (3H, s, 5-OCH_3), 5.75 (1H, q, 6.5 Hz, H-3), 6.92 (1H, d, 8.0 Hz, H-6), 7.39 (1H, dd, 8.0 Hz, 8.0 Hz H-7), 7.55 (1H, d, 8.0 Hz, H-8), 7.86 (1H, s, H-9).

^{13}C-NMR (125 MHz, CD_3OD) (δ ppm): 170.5 (O-C=O), 156.5 (C-5), 149.1 (C-4), 137.2 (C-9a), 127.9 (C-3a), 126.5 (C-7), 125.8 (C-4a), 123.6 (C-8), 117.5 (C-8a); 116.4 (C-9), 106.2 (C-6), 77.1 (C-3), 56.4 (C-11), 19.1 (C-10).

Compound **2** (resveratrol): White needle crystals, m.p. 261-263 ^{o}C. $C_{14}H_{12}O_3$. ESI-MS *m/z* 229 $[M+H]^+$.

^{1}H-NMR (500 MHz, CH_3OD), (δ ppm): 6.19 (1H, t, 2.0 Hz, H-4), 6.47 (2H, d, 2.5 Hz, H-2, 6), 6.78 (2H, d, 9.0 Hz, H-3',5'), 6.81 (1H, d, 16. 5 Hz, H-*β*), 6.97 (1H, d, 16. 5 Hz, H-*α*); 7.36 (2H, d, 8.5 Hz, H-2', 6').

^{13}C-NMR (125 MHz, CH_3OD) (δ ppm): 159.6 (C-3, 5), 158.3 (C-4'), 141.3 (C-1), 130.4 (C-1'), 129.4 (C-*α*), 128.8 (C-2', 6'), 127.0 (C-*β*), 116.5(C-3', 5'), 105.8 (C-2, 6); 102.6 (C-4).

Compound **3** (polydatin)**:** White needle crystals, m.p. 223-226 ^{o}C. $C_{20}H_{22}O_8$. ESI-MS: *m/z* 413 $[M+Na]^+$.

^{1}H-NMR (500 MHz, CD_3OD), (δ ppm): 3.40 - 3.43 (2H, m, H-6", 6"b), 3.45 - 3.52 (2H, m, H-3", 4"), 3.74 (1H, dd, 6.0 Hz; 12.0 Hz H-5") 3.95 (1H, dd, 2.0 Hz; 12.0 Hz H-2"), 4.91 (1H, d, 7.5 Hz, H-1"), 6.47 (1H, t, 2.0 Hz, H-4), 6.64 (1H, s, H-6), 6.78 (2H, d, 8.5 Hz, H-3',5'), 6.80 (1H, s, H-2), 6.86 (1H, d, 16.0 Hz, H-*β*), 7.03 (1H, d, 16.0 Hz, H-*α*), 7.38 (2H, d, 8.5 Hz, H-2', 6').

^{13}C-NMR (125 MHz, CH_3OD) (δ ppm): 160.4 (C-3) 159.5 (C-5), 158.4 (C-4'), 141.4 (C-1), 130.3 (C-1'), 129.9 (C-*α*), 128.9 (C-2', 6'), 126.6 (C-*β*), 116.5 (C-3', 5'), 108.3 (C-6), 107.0 (C-2), 104.4 (C-4), 102.4 (C-1"), 78.2 (C-5"), 78.0 (C-3"), 74.9 (C-2"), 71.5 (C-4"), 62.6 (C-6").

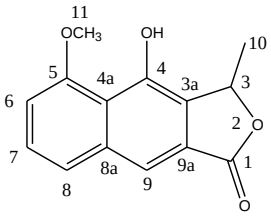
Compound **4** *(β*-sitosterol): Colourless needle crystals, m.p. 137 – 139 °C.

IR υ*max (KBr, cm-1): 3429 (OH); 2939; 1663 (>C=C<); 1468; 1381; 1051 cm-1 (C-O).

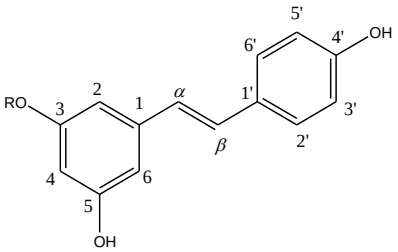
Compound **5** (*β*-sitosterol-3-O-*β*-D-glucopyranoside): White powder, m.p. 285 – 286 ^{o}C.

3. RESULTS AND DISCUSSION

Our study on the ethyl acetate extract of fruits of *Dipterocarpus retusus* led to the isolation of five compounds (**1**-**5**).

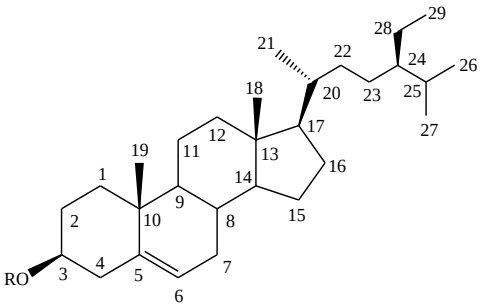


1. eleutherol



2. *trans*- resveratrol R = H

3. polydatin R = *β*-D-glucopyranosyl



4. *β* - sitosterol R= H

5. *β* - sitosterol-3-O-*β*-D-glucopyranoside R= *β*-D-glucopyranosyl

The ^{1}H-NMR spectrum of compound **1** showed signals of 12 protons. Three signals of aromatic protons at δ_H 6.92 ppm (1H, d, *J* = 8.0 Hz, H-6), 7.39 ppm (1H, dd, *J* = 8.0 Hz, 8.0 Hz, H-7), 7.55 ppm (1H, d, *J* = 8.0 Hz, H-8), indicative of a 1,2,3-trisubstituted benzene ring; and a single aromatic proton at δ_H 7.86 ppm (1H, s, H-9), combined with signals of 10 aromatic carbons (between 106.0 and 156.5 ppm) suggested the presence of a tetrasubstituted naphthalene ring skeleton. Moreover, it showed the presence of one methyl group at δ_H 1.72 ppm (3H, d, *J* = 6.5 Hz, 3-CH_3), one methine proton at δ_H 5.75 ppm (1H, q, *J* = 6.5 Hz, H-3), and one methoxyl group at δ_H 4.13 ppm (3H, s, 5-OCH_3) with one hydroxy proton at δ_H 9.63 ppm (1H, s, 4-OH) bonded to aromatic ring. The ^{13}C-NMR spectrum of compound **1** showed signals of 14 carbons including 10 aromatic carbons, one methoxy carbon at δ_C 56.4 ppm (C -11), one methyl carbon at δ_C 19.1 ppm (C -10), one carboxyl carbon at δ_C 170.5 ppm (C-1), and one oxygenated carbon at δ_C 77.1 ppm (C-3) connected to the oxygen atom of a five-membered-ring lactone. In the lower field of the ^{13}C-NMR spectrum, there are two signals at δ_C 156.5 ppm (C-5) and 149.1 ppm (C-4) corresponding to two oxygenated aromatic carbons. This analysis, combined with HSQC and HMBC spectra data of compound 1 and the comparison with reference data [8] confirmed that compound **1** is eleutherol.

The ^{1}H-NMR spectrum of compound **2** presenting signals of 7 aromatic protons at δ_H 6.19 ppm (1H, t, *J* = 2.0 Hz, H-4), 6.47 ppm (2H, d, *J* = 2.5 Hz, H-2, 6) and 6.78 ppm (2H, d, *J* = 9 Hz, H-3', 5'), 7.36 ppm (2H, d, *J* = 8.5 Hz, H-2', 6'), together with two *trans*-coupling olefinic protons at δ_H 6.81 ppm (1H, d, *J* = 16.5 Hz, H-*β*) and 6.97 ppm (1H, d, *J* = 16.5 Hz, H-*α*) are characteristics of *trans*-resveratrol. In the lower field of the ^{13}C-NMR spectrum, there are two signals at δ_C 159.6 ppm (C-3, 5) and 158.3 ppm (C-4') corresponding to three oxygenated aromatic carbons. This analysis, combined with HSQC and HMBC spectra data and the comparison with references [9-11] confirmed that the compound **2** is 3,5,4'-trihydroxy-*trans*–stilbene (*trans*- resveratrol).

The NMR spectra of compound **3** are similar to those of compound **2**, except that the spectrum of compound **3** showed a set of signals presenting a *β*-D-glucopyranosyl unit at chemical shifts between δ_H 3.0 ppm and 5.0 ppm in ^{1}H-NMR and δ_C 60 ppm and 102 ppm in ^{13}C-NMR spectra. In the down field of the ^{1}H-NMR spectrum, there are only two singlet signals of aromatic protons at δ_H 6.64 ppm (1H, s, H-6), 6.80 ppm (1H, s, H-2). The comparison with those of compound **2** suggested that the hydroxyl group at C-3 was replaced by a glucose unit. The comparison with reference data [12] confirmed compound **3** to be 5,4'-dihydroxy stilbene-3-O-*β*-D-glucopyranoside (**polydatin**).

It is the first time that compounds **1**, **2** and **3** have been found from the fruits of *D. retusus* Blume.

The structures of compound **4** and **5** were identified by comparing physicochemical and spectroscopic data to the published values for *β*-sitosterol and *β*-sitosterol-3-O-*β*-D-glucopyranoside [13].

Table 1. *In vitro* cytotoxic activity and antioxidant activity of the extracts and isolated compounds of *D. retusus* Blume.

№	Test samples	IC_{50} (μg/ml)	EC_{50} (μg/ml)
		KB	**DPPH**
1	Ethanol extract	>256	>256
2	*n*-hexane extract	81.06	>256
3	EtOAc extract	34.46	246
4	BuOH extract	40.38	>256
5	Compound **1**	>256	>256
6	Compound **3**	>256	>256
7	**Ellipticine**	**0.21**	
7	**Quercetin**		**8,1**

The different extracts (ethanol, n-hexan, ethyl acetate, buthanol) of fruits of *Dipterocarpus retusus* Blume were tested for antioxidant and cytotoxic activities on KB cell line. The results are shown in Table 1.

The results showed that all three extracts exhibited the cytotoxic activity against KB cell line, in which ethyl acetate extract exhibited the strongest activity with IC_{50} values of 34.46 μg/ml, while two compounds **1** and **3** did not show activity against KB cell line. Compounds **1** and **3** were reported to have antifungal and antioxidant activity [14, 15]. However, all the extracts and compounds **1** and **3** did not show antioxidant activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.

4. CONCLUSIONS

This is the first report on the isolation of eleutherol (**1**), *trans*- resveratrol (**2**), polydatin (**3**), *β*-sitosterol (**4**), and *β*-sitosterol-3-O-*β*-D-glucopyranoside (**5**) from the fruits of *Dipterocarpus retusus* Blume. The chemical structures of the isolates were elucidated based on NMR spectroscopy and compared with literature data. The *n*-hexane, ethyl acetate and butanol extracts of the fruits of *D. retusus* showed cytotoxic activities against KB cell line.

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