FLAVONOIDS FROM PODS OF *Vigna radiata* (L.) Wilczeck

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Le Thi Huyen*, Nhu Thi Hang Nga
VNU University of Science, Hanoi, 334 Nguyen Trai, Thanh Xuan, Hanoi, Vietnam.
*Email: lethihuyen@hus.edu.vn

TÓM TẮT

CÁC HỢP CHẤT FLAVONOID PHÂN LẬP TỪ VỎ ĐẬU XANH

Bốn hợp chất flavonoid, vitexin (1), isovitexin (2), isoorientin (3), taxifolin (4), và một hợp chất steroid daucosterol (5) đã được phân lập từ dịch chiết methanol của phần vỏ đậu xanh. Cấu trúc của các chất này được xác định dựa trên phân tích các dữ kiện phổ khối lượng, phổ cộng hưởng từ hạt nhân một chiều và hai chiều, và kết hợp so sánh với các dữ liệu phổ đã công bố. Ba hợp chất 3-5 lần đầu tiên được phân lập từ vỏ đậu xanh.

***Từ khóa**: Vigna radiata, flavonoid, isoorientin, taxifolin, daucosterol.*

1. INTRODUCTION

Vigna radiata (L.) Wilczek, commonly known as mung bean, is a legume cultivated for its edible seeds and sprouts across Asia. South Asia produces over 80% of the world's mung beans [1]. Mung bean is also grown in China, Australia, and the United States of America [2-4]. In Vietnam, mung bean is grown throughout the country from North to South. This is an important vegetable, food, and a valuable bean in Vietnamese culinary culture. Mung bean is also well known for its bioactive compounds, including proteins, phenolics, and flavonoids with various health benefits [5]. Most by-products from the mung bean industry are mung bean pods, which are usually discarded. In addition, phenolics and flavonoids were found to be abundant in mung bean pods at 84.2% and 83.9%, respectively [6]. Mung bean pod extracts also exhibited antioxidant, antimicrobial, anti-inflammatory, and antitumor activities [7-10]. In this study, we report the isolation and structural elucidation of four flavonoids and one steroid from the ethyl acetate extract of *V. radiata* pods.

2. EXPERIMENTAL

2.1. Plant materials

The dried pods of *Vigna radiata* (L.) Wilczek were collected in Ha Nam, Viet Nam, in Jun 2022 and were deposited at the Lab of Pharmaceutical Chemistry, VNU University of Science, Hanoi.

2.2. Experimental equipment

All NMR spectra, including ^{1}H-NMR (500 MHz), ^{13}C (125 MHz), HSQC, and HMBC, were recorded on a Bruker AM500 FT-NMR spectrometer, and TMS was used as an internal standard. Optical rotations were determined on a Jasco DIP-370 automatic polarimeter. Column chromatography (CC) was performed using a silica gel (Kieselgel 60, 70-230 mesh, and 23,0-400 mesh, Merck) or RP-18 resins (30-50 µm, Fuji Silysia Chemical Ltd.). Thin layer chromatography (TLC) was done using pre-coated silica gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck).

2.3. Extraction and separation

Mung bean pods are separated, dried, and ground into powder. The dried powdered pods of *V. radiata* (4.1 kg) were ultrasonically extracted with methanol (MeOH) three times (each 10 L, 5h, 25ºC). After removal of the solvent, the MeOH extract was suspended in water and successively partitioned with *n*-hexane, dichloromethane (CH_2Cl_2), and ethyl acetate (EtOAc) to give the corresponding *n*-hexane (N,120g), dichloromethane (D, 156g), EtOAc (E, 238g) residues, and water layer (W). The E fraction was carried out by a silica gel column eluting with gradient solvent system CH_2Cl_2/MeOH (100/1, 99/1, 49/1, 19/1, 9/1, 9/1, v/v) to yield sixteen fractions E1–E16. The E15 fraction was loaded on a Sephadex LH-20 CC eluting with MeOH/water (3/1, v/v) to provide eight fractions, E15A-E15H. The E15E fraction was chromatographed on an RP -18 CC eluting with acetone/water (1/1, v/v) to provide five fractions, E15E1-E15E5. E15E3 fraction was loaded on a Sephadex LH-20 CC eluting with MeOH/water (3/1, v/v) to provide four fractions, E15E3A-E15E3D. The E15E3C fraction was run on an RP-18 CC eluting with acetone/water (1/2,5, v/v) to yield compound **2**. The E15E3C2 fraction was applied on an RP - 18 CC eluting with acetone/water (1/2.5, v/v) to obtain compound **1**. The E5 fraction (3 g) was chromatographed on a Sephadex LH-20 column and eluted with MeOH/water (3/1, v/v) to obtain six fractions E5A - E5F. The fraction E5B was crystallized in a mixture solvent of *n*-hexane/EtOAc (8/2, v/v), yielding compound **3** (12 mg). The E7 fraction (20 g) was separated on a silica gel column eluting with a gradient solvent system of *n*-hexane/EtOAc to get eight sub-fractions labeled E7A-E7H. The E7C fraction (80 mg) was separated on a Sephadex LH-20 column and eluted with MeOH/water (3/1, v/v) to obtain smaller fractions E7C1-E7C4. The E7C1 fraction was further separated on a silica gel column eluting with dichloromethane-ethanol (2.0/1, v/v) to afford compound **4** (5.0 mg). The D fraction was carried out on a silica gel column eluting with CH_2Cl_2/MeOH (100/1, 99/1, 49/1, 19/1, 9/1, 9/1, v/v) to yield ten fractions D1– D10. Finally, the D4 fraction was fractionally crystallized in *n*-hexane to obtain compound **5.**

Vitexin (1): yellow powder; ESI-MS *m/z* 433 $[M+H]^+$, $C_{21}H_{20}O_{10}$; ^{1}H- and ^{13}C-NMR (DMSO): see Table 1.

Isovitexin (2): yellow powder; ESI-MS *m/z* 433 $[M+H]^+$, $C_{21}H_{20}O_{10}$; ^{1}H- and ^{13}C-NMR (DMSO): see Table 1.

Isoorientine (3): pale-yellow powder; ESI-MS *m/z* 449 $[M+H]^+$, $C_{21}H_{20}O_{11}$; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 1.

Taxifolin (4): pale-yellow needle; ESI-MS *m/z* 305 $[M+H]^+$, $C_{15}H_{12}O_7$, ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 1.

Daucosterol (5): white needle;

^{1}H NMR: *δ (ppm)*: 5.32 (1H, m, H-6), 4.99 (1H, d, *J* = 7.7 Hz, H-1′), 4.50 (1H, dd, *J* = 11.6 Hz, 2.1 Hz,H-6′*β*), 4.36 (1H, dd, *J* = 11.7 Hz, 5.2 Hz, H-6′*α*), 4.23 (2H, m, H-3′, H-4′), 4.00 (1H, t, *J* = 7.9 Hz, H-2′), 3.93 (1H, m, H-5′), 3.90 (1H, m, H-3), 0.96 (3H, d, *J* = 6.4 Hz, Me-21), 0.91 (3H, s, Me-19), 0.87(3H, t, *J* =7.3Hz, Me-29), 0.85 (3H, d, *J* = 6.8Hz, Me-26), 0.83 (3H, d, *J* = 6.9 Hz, Me-27), 0.64 (3H, s, Me-18);

^{13}C NMR: *δ (ppm)*: 38.8 (C-1), 33.5 (C-2), 79.8 (C-3), 41.3 (C-4), 142.2 (C-5),123.2 (C-6), 31.6 (C-7), 33.4 (C-8), 51.7 (C-9), 38.2 (C-10), 22.7 (C-11), 40.7 (C-12), 43.8 (C-13), 58.1 (C-14), 25.8 (C-15), 29.9 (C-16), 57.6 (C-17), 13.5 (C-18), 20.7 (C-19), 37.7 (C-20), 20.3 (C-21), 35.5 (C-22), 27.7 (C-23), 47.4 (C-24), 30.7 (C-25), 21.3 (C-26), 20.5 (C-27), 24.7 (C-28), 13.3 (C-29), 103.9 (C-1′), 76.7 (C-2′), 79.9 (C-3′),73.0 (C-4′), 79.4 (C-5′), 64.1 (C-6′).

3. RESULTS AND DISCUSSION

3.1. Structural elucidation

Compound **1** was obtained as a yellow amorphous powder. Its ESI-MS had a pseudo molecular ion peak at *m/z* 431.09 $[M-H]^-$, calculating a molecular formula of $C_{21}H_{20}O_{10}$.

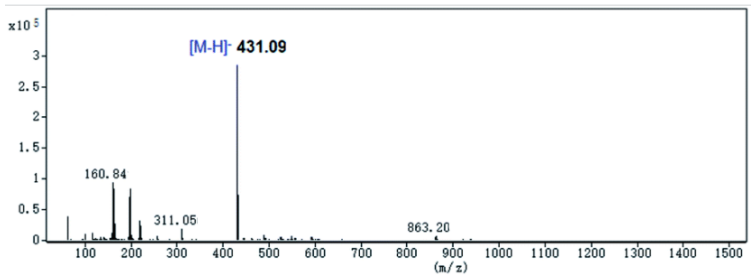

*Figure 1. ESI-MS of compound **1***

The ^{1}H-NMR spectrum of **1** showed the signals of four aromatic protons of an A_2B_2 coupled system at δ_H 8.02 (2H, d, J = 8.5 Hz, H-2′, H-6′) and δ_H 6.88 (2H, d, J = 8.5 Hz, H-3′, H-5′), indicating the *para*-substituted in the B ring. Two singlet aromatic protons at δ_H 6.77 (1H, s, H-3) and 6.27 (1H, s, H-6), and singlet signal at δ_H 13.16 specific for intramolecular hydrogen bonding at the C-5 position were also observed in the ^{1}H-NMR spectrum.

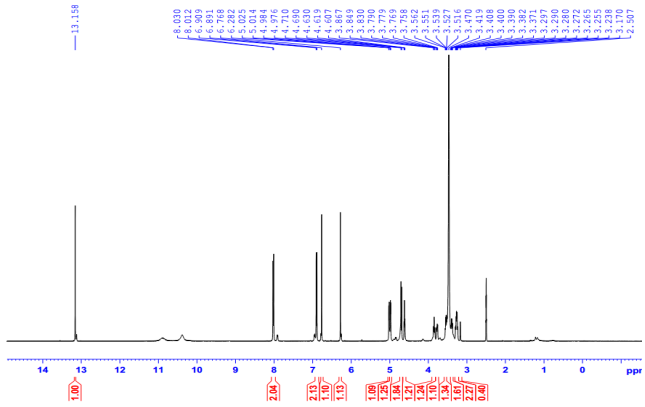

*Figure 2. ^{1}H-NMR spectrum of compound **1***

Moreover, typical signals due to a glucopyranosyl moiety were observed at δ_H 4.80 (1H, d, J = 10.0 Hz, H-1") and hydroxymethylene at 3.78 (dd, 5.0, 12.0) and 3.88 (dd, 2.0, 12.0), of which the large coupling constant J = 10.0 Hz of anomeric proton indicated the β-linkage with the aglycone. The ^{13}C-NMR and DEPT spectra (Table1) revealed signals of 21 carbons, including 15 carbons of an aglycone and six carbons of a glucopyranosyl unit at δ_C 81.8, 78.7, 73.4, 70.9, 70.6, and 61.4. Analysis of ^{1}H- and ^{13}C-NMR data suggested that **1** was an 8-C-glycosyl apigenin structure (Table 1).

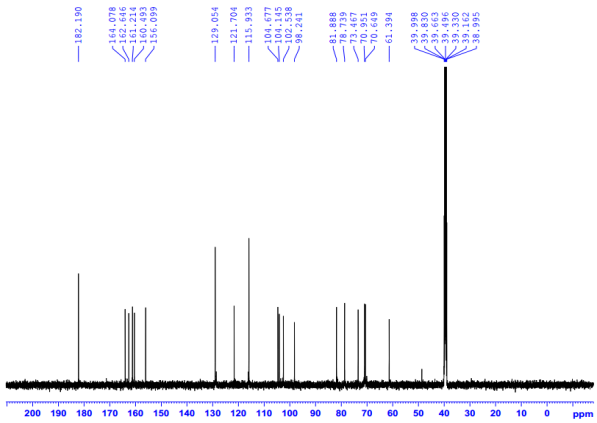

*Figure 3. ^{13}C-NMR spectrum of compound **1***

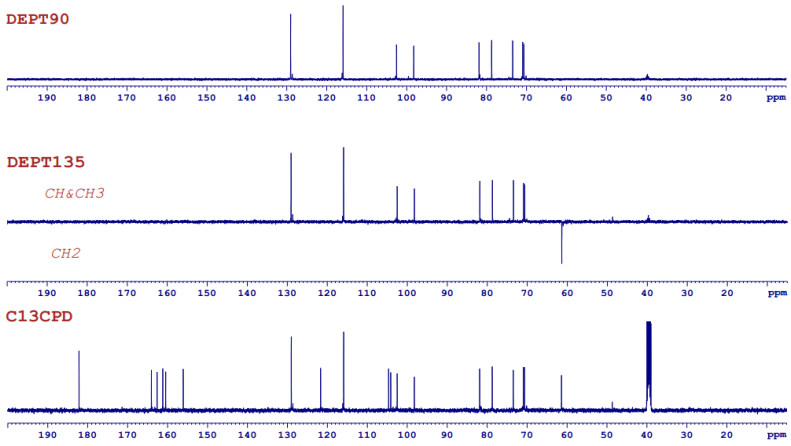

*Figure 4. DEPT spectrum of compound **1***

The assignment of all carbons and protons and, thereby, the structure of the compound was resolved by 2D experiments, notably HSQC and HMBC experiments. The location of a glucopyranosyl unit determined at C-8 of an apigenin skeleton was proved by the HMBC correlations observed from H-1" (δ_H 4.80) to C-8 (δ_C 104.0), as shown in Figure 8.

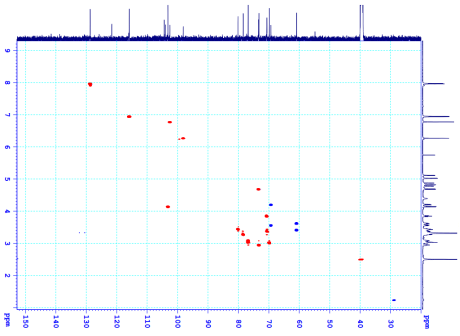

*Figure 5. HSQC spectrum of compound **1***

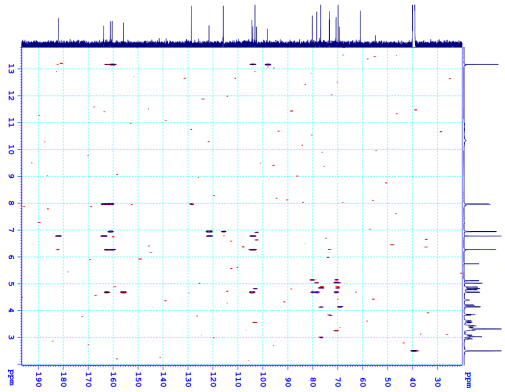

*Figure 6. HMBC spectrum of compound **1***

Its NMR data matched perfectly with those reported in the literature [11]. The structure of **1** was therefore identified as vitexin, a compound previously isolated from mung bean pods.

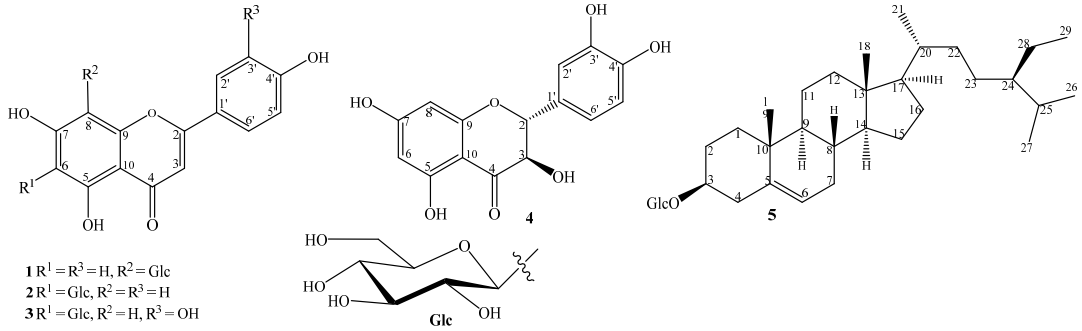

*Figure 7. Chemical structure of compounds **1-5***

Table 1. ^{1}H- and ^{13}C-NMR data of compounds 1-4

C		**1**		**2**		**3**		**4**	
		δ_C[a,b]	δ_H[a, d] (mult, *J* in Hz)	δ_C[a,b]	δ_H[a, d] (mult, *J* in Hz)	δ_C[c,b]	δ_H[c, d] (mult, *J* in Hz)	δ_C[c,b]	δ_H[c, d] (mult, *J* in Hz)
Aglycone	2	164.0	-	166.1	-	163.3	-	85.1	4.52 (d, 11.5)
	3	102.5	6.77 (s)	103.8	6.56 (s)	102.8	6.47 (s)	73.7	4.93 (d, 11.5)
	4	182.1	-	184.0	-	181.9	-	198.4	-
	5	160.4	-	162.0	-	160.7	-	168.8	-
	6	98.2	6.27 (s)	109.1	-	108.9	-	97.3	5.94 (d, 2.0)
	7	162.7	-	164.8	-	163.7	-	165.3	-
	8	104.0	-	95.3	6.48 (s)	93.5	6.66 (s)	96.3	5.91 (d, 2.0)
	9	156.0	-	158.6	-	156.2	-	164.5	-
	10	104.6	-	105.2	-	103.4	-	101.8	-
	1′	121.6	-	123.0	-	121.4	-	129.9	-
	2′	129.0	8.02 (d, 8.5)	129.4	7.81 (d, 8.5)	113.3	7.41 (d, 2.0)	115.9	6.99 (d, 1.5)
	3′	115.9	6.88 (d, 8.5)	117.0	6.92 (d, 8.5)	145.8	-	147.1	-
	4′	161.2	-	162.7	-	149.7	-	146.3	-
	5′	115.9	6.88 (d, 8.5)	117.0	6.92 (d, 8.5)	116.1	6.89 (d, 8.0)	116.1	6.82 (8.0)
	6′	129.0	8.02 (d, 8.5)	129.4	7.81 (d, 8.5)	119.0	7.41 (dd, 8.0, 2.0)	120.9	6.87 (dd, 1.5; 8.0)
Glycoside	1″	73.4	4.80 (d, 10.0)	75.3	4.92 (d, 10.0)	73.1	4.59 (d, 10.0)		
	2″	70.9	3.83 (t, 9.5)	72.6	4.20 (t, 8.0)	70.6	4.04 (t, 9.0)		
	3″	78.7	3.29 (m)	80.1	3.50 (m)	78.9	3.42 (m)		
	4″	70.6	3.34 (m)	71.8	3.51 (m)	70.2	3.43 (m)		
	5″	81.8	3.25 (m)	82.6	3.46 (m)	81.5	3.45(m)		
	6″	61.4	3.68 (dd, 5.0, 12.0) 3.88 (dd, 2.0, 12.0)	62.9	3.77 (dd, 5.0, 12.0) 3.91 (dd, 2.0, 12.0)	61.5	3.68 (d, 11.5) 3.89 (dd, 11.5, 5.5)		

Measured in [a] CD_3OD, [b] 125MHz, [c] 500MHz

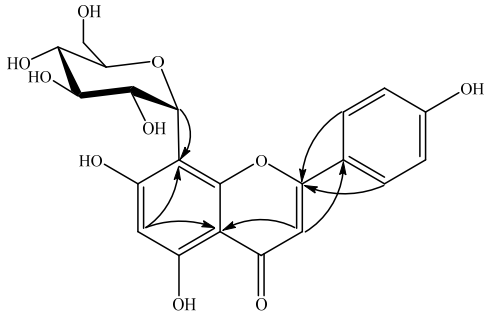

*Figure 8. The key HMBC correlations of compound **1***

Compound **2** was isolated as a yellow powder. It had a pseudo molecular ion peak at *m/z* 433 $[M+H]^+$ in the ESI-MS, predicting a molecular formula of $C_{21}H_{20}O_{10}$ as compound **1**. The ^{1}H-

and ^{13}C-NMR spectral data of compound **2** were very similar to those of compound **1,** suggesting that 1 is also a flavone C-glycoside. In addition, the same molecular between **1** and **2** indicated that they are isomers. The difference might be in the location of glucopyranosyl moiety at the apigenin skeleton; instead of locating at C-8 of **1**, the sugar unit attached to C-6 of the aglycone. Moreover, a comparison of the ^{1}H- and ^{13}C-NMR data of **2** with reported data in the literature for isovitexin showed excellent agreement [12]. Thus, compound **2** was characterized as isovitexin.

Compound **3** was obtained as a yellow solid. It possessed a molecular formula of $C_{21}H_{20}O_{11}$ as demonstrated from the ESI-MS spectrum at *m/z* 449 $[M+H]^+$ and ^{13}C-NMR data. The ^{1}H-NMR spectrum of **3** showed signals of ABX spin systems of a tri-substituted benzene ring at [δ_H 6.89 (d, J = 8.0 Hz), 7.41 (d, J = 2.0 Hz), 7.41 (dd, J = 8.0, 2.0 Hz)]; two singlet aromatic proton signals at δ_H 6.47 and 6.66; and one anomeric proton at δ_H 4.59 (d, J = 10.0 Hz). The ^{13}C-NMR and HSQC spectrum of **3** showed the signals of 21 carbons, including one carbonyl group at δ_C 181.9, fourteen aromatic carbons at δ_C 93.5 to 163.3, and six carbons of a sugar moiety. In addition, the signals of C-glucopyranoside were proved based on the appearance of the characteristic signals on the ^{1}H-NMR spectrum at δ_H 4.59 (d, J = 10.0 Hz), hydroxymethylene at δ_H 3.68 (d, J = 11.5 Hz) and 3.89 (dd, J = 11.5, 5.5 Hz), and the ^{13}C-NMR spectra at δ_C 61.5, 70.2, 70.6, 73.1, 78.9, and 81.5 ppm. The β-configuration for the anomeric carbon of glucose was suggested by the large coupling constant (10.0 Hz) of H-1' (δ_H 4.59). Analysis of ^{1}H- and ^{13}C-NMR data indicated that **3** was an 8-C-glycosyl luteolin structure (Table 1). Comparing 1D NMR data of **3** with those published in the literature [13] led to identifying **3** as isoorientin, a compound previously found in some plans such as *Aspalathus linearis*, *Aloe vera, Annona muricata* [13].

Compound **4** was obtained as a pale-yellow needle. Its molecular formula was predicted to be $C_{15}H_{12}O_7$ based on a quasi-molecular ion peak at *m/z* 305 $[M+H]^+$ in the ESI-MS and consistent with ^{13}C-NMR data. The ^{1}H-NMR spectra of compound **4** indicated the signal of three aromatic protons of an ABX coupled type at δ_H 6.99 (1H, d, J = 2.0 Hz, H-2'), 6.87 (1H, dd, J = 2.0, 8.5 Hz, H-6'), 6.87 (1H, d, J = 8.5 Hz, H-5') and two *meta*-coupled aromatic protons at δ_H 5.93 (1H, d, J = 1.5 Hz, H-8) and 5.90 (1H, br s, H-6). In addition, two other oxymethine resonances at δ_H 4.92 (1H, d, J = 11.5 Hz, H-3); 4.51 (1H, d, J = 11.5 Hz, H-2) suggested that it might be a dihydroflavonol. The ^{13}C-NMR spectra of compound **4** revealed signals of 15 carbons, including one carbonyl group at δ_C 198.2, five methines at δ_C 96.3, 73.3, 115.9, 116.1, 120.9, two quaternary carbons at 101.8, 129.9, five O-bearing C-atoms at δ_C 146.3, 147.1, 164.5, 165.3, 168.8, and two oxymethines at δ_C 85.1 (C-2), 73.6 (C-3). Moreover, a comparison of the NMR data of compound **4** with those of the reference compound [14] led to the elucidation of the structure of compound **4** as taxifolin, a compound previously isolated from flowers of *Musa spp. (Baxijiao)* [14].

Compound **5** was obtained as a white amorphous powder. The structure was identified as daucosterol by extensively analyzing its ^{1}H- and ^{13}C-NMR data and comparison with previous data [15].

4. CONCLUSION

Four flavonoids, vitexin (1), isovitexin (2), isoorientin (3), taxifolin (4), together with one steroid daucosterol (5), were isolated from the methanol extract of the pods of *Vigna radiata* (L.) Wilzeck using combined chromatographic methods. Of these, three compounds 3-5 has been isolated for the first time from the pods of *V. radiata;* vitexin (1) and isovitexin (2) were identified to be the two major flavonoids in the mung bean pods [5, 16].

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