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PHENOLIC CONSTITUENTS FROM THE ETHYL ACETATE EXTRACT OF *CAMELLIA FLAVA* FLOWERS (THEACEAE) COLLECTED IN PHU THO PROVINCE

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Summary

Camellia flava (Pitard) Sealy is one of the yellow camellia species belonging to the genus *Camellia* (family Theaceae), which is widely distributed in Vietnam. Phytochemical investigation of the ethyl acetate extract of *C. flava* flowers collected in Phu Tho province led to the identification of eight phenolic compounds, including 3,3',4'-tri-*O*-methyl ellagic acid (**1**), pinoresinol (**2**), quercetin (**3**), kaempferol (**4**), tyrosol (**5**), caffeic acid (**6**), protocatechuic acid (**7**), and gallic acid (**8**). The structures of the isolated compounds were determined by NMR and MS spectroscopic analyses and comparison with the reported data. This study represents the first report of compounds (**1**–**8**) from the flowers of *C. flava*.

Keywords: *Camellia flava; Camellia cucphuongensis; Flavonoid; Lignan; Phenolic.*

1. Introduction

Yellow Camellias, belonging to the genus *Camellia* L. (family Theaceae), represent a distinct group of plants characterized by their unique yellow flowers. Unlike the traditional green tea *Camellia sinensis*, yellow Camellias have remained poorly studied until recent decades, when their exceptional ornamental value and potential medicinal properties began attracting scientific attention [1]. The genus *Camellia* comprises approximately 300 accepted species, with about 97 species distributed in China and 95 species and 2 varieties recorded in Vietnam. Among these, around 69 yellow *Camellia* species have been described, of which over 50 species are found in Vietnam [2]. In recent years, phytochemical studies have revealed that yellow Camellias contain diverse classes of bioactive compounds comparable to those found in *C. sinensis*, including flavonoids, phenolic compounds, saponins, triterpenoids, phytosterols, essential oils, polysaccharides, and amino acids [1]. However, chemical investigations have focused primarily on a few species like *C. nitidissima, C. chrysantha*, and *C. bugiamapensis*, with *C. nitidissima* receiving the most intensive research attention [1],[3]. These studies have demonstrated that extracts and isolated compounds from yellow Camellias possess a wide spectrum of biological activities, including antioxidant, anticancer, hypolipidemic, hypoglycemic, hepatoprotective, neuroprotective, and antidepressant effects [3].

Camellia flava (Pitard) Sealy (synonym: *Camellia cucphuongensis* Ninh & Rosmann), one of the yellow *Camellia* species, is mainly distributed in Ninh Binh and Phu Tho provinces. However, research on this species remains limited. Nguyen et al. have studied the anti-diabetic and hypolipidemic effects of *C. flava* flower and leaves extracts [4],[5],[6],[7]. The high concentration of polyphenols, total flavonoids, and antioxidant capacity of *C. flava* leaves were also reported by this group [8]. However, only one isolation study of *C. flava* leaves has been reported by Do et al., which identified phenolic compounds including kaempferol, gallic acid, astragalin, and vanillin [9]. As part of our efforts to discover bioactive compounds from medicinal plants, in this current study, we aimed to isolate and structurally elucidate chemical constituents from the ethyl acetate extract of unexplored *C. flava* flowers collected in Phu Tho province.

2. Materials and Methods

2.1. Materials

Flowers of *Camellia flava* (Pitard) Sealy (Theaceae) were provided by Biopharm Hoa

Binh Joint Stock Company in Hoa Binh ward, Phu Tho province, in October 2024. The plant material was identified by Prof. Tran The Bach, Institute of Biology, Vietnam Academy of Science and Technology (VAST). The voucher specimen (Cong21102024) was deposited at the Institute of Biology (VAST).

2.2. General experimental procedures

Solvents and chemicals used for extraction and isolation were of analytical grade. The stationary phases for column chromatography included *silica gel* (240–430 mesh, Merck), reversed-phase *silica gel* (RP-18 YMC), and Sephadex LH-20 (Sigma). Thin-layer chromatography was performed on pre-coated *silica gel* plates (Merck 60 F_{254}). Visualization was carried out under a UV lamp at 254 nm or by spraying with 10% sulfuric acid reagent followed by heating. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker 600 MHz and Bruker AM500 FT-NMR spectrometers. Mass spectra (ESI-MS) were measured using an Agilent 1260 LC/MS system.

2.3. Extraction and isolation

The dried flower powder (2.6 kg) was macerated with 70% EtOH at room temperature for 24 hours (13 L × 4 times). The combined EtOH extracts were concentrated under reduced pressure to afford an EtOH residue (1.167 kg). This residue was dissolved in 500 mL of distilled water and successively partitioned with *n*-hexane and ethyl acetate (EtOAc). Removal of the solvent from the EtOAc fraction yielded an EtOAc residue (98.5 g).

The EtOAc residue was subjected to *silica gel* column chromatography (CC), and eluted with a gradient of *n*-hexane–EtOAc (0–100%) to afford nine fractions E1–E9. Fraction E6 (269 mg) was separated by Sephadex LH-20 CC, eluted with MeOH, yielding 7 fractions (E6A–E6G). Fraction E6A (53 mg) was purified by reversed-phase *silica gel* CC eluting with acetone–water (3:1, v/v) to obtain **4** (5.5 mg). Fraction E6D (46 mg) was purified by reversed-phase *silica gel* CC and eluted with acetone–water (2:1, v/v) to obtain **5** (3.4 mg). Fraction E7 (1.45 g) was separated on a *silica gel* column, eluting with *n*-hexane–acetone (8:2, v/v) to afford 10 fractions (E7A–E7K). Fraction E7D (53 mg) was purified by reversed-phase *silica gel* CC, eluting with acetone–water (2:1, v/v) to give **7** (4.5 mg). Fraction E7K (112 mg) was chromatographed by reversed-phase *silica gel* CC using acetone–water (2:1, *v/v*) as eluent to afford **3** (3.4 mg). Fraction E7F (36 mg) was separated by reversed-phase *silica gel* CC eluting with acetone–water (2:1, v/v) to yield **6** (2.4 mg).

Fraction E8 (1.69 g) was separated on a *silica gel* CC eluting with *n*-hexane–acetone (8:2, v/v) to afford 9 fractions (E8A–E8I). Compound **1** (5.6 mg) was obtained by recrystallization in MeOH from fraction E8A. Fraction E8G (245 mg) was purified by reversed-phase *silica gel* CC eluting with acetone–water (2:1, v/v) to obtain **2** (4.5 mg). Fraction E8I (123 mg) was purified by reversed-phase *silica gel* CC and eluted with MeOH–water (2:1, *v/v*) to give **8** (6.5 mg).

3,3',4'-Tri-O-methyl ellagic acid (1): White solid. ESI-MS *m/z* 345 $[M+H]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6): see Table 1.

Pinoresinol (2): White solid. ESI-MS *m/z* 357 $[M-H]^-$. ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): See Table 1.

Quercetin (**3**): Pale yellow solid. ESI-MS *m/z* 303.1 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): see Table 2.

Kaempferol (4): Pale yellow solid. ESI-MS *m/z* 287.1 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): see Table 2.

Tyrosol (5): White solid. ^{1}H-NMR (600 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6): see Table 3.

Caffeic acid (6): Pale yellow solid. ESI-MS *m/z* 179.1 $[M-H]^-$. ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): See Table 3.

Protocatechuic acid (7): Brown solid. ^{1}H-NMR (600 MHz, CD_3OD) δ (ppm): 7.44 (1H, br s, H-2), 7.42 (1H, d, J = 8.4 Hz, H-6), 6.79 (1H, d, J = 8.4 Hz, H-5). ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 169.5 (COOH), 149.7 (C-4), 144.0 (C-3), 123.3 (C-1), 121.6 (C-6), 116.5 (C-5), 114.6 (C-2).

Gallic acid (8): Yellow solid. ^{1}H-NMR (600 MHz, CD_3OD) δ (ppm): 7.07 (2H, s, H-2, H-6). ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 170.3 (COOH), 146.3 (C-4), 139.6 (C-3, C-5), 110.3 (C-2, C-6), 121.9 (C-1).

3. Results and Discussion

Compound **1** was isolated as a white solid. The ^{1}H-NMR spectrum showed two signals in the aromatic region at δ_H 7.49 (s, H-5) and 7.57 (s, H-5'); 3 methoxy group signals at δ_H 4.05 (3H, s), 4.03 (3H, s), and 3.98 (3H, s). The ^{13}C-NMR spectrum revealed the signals of 17 carbons, including two carboxyl carbon signals at δ_C 158.5 (C-7') and 158.4 (C-7); 12 aromatic carbons at δ_C 153.8, 152.9, 141.9, 141.0, 140.8,

140.3, 113.4, 112.5, 111.8, 111.8, 111.0, and 107.5 and 3 methoxy group signals at δ_C 61.3, 61.0, and 56.7. The ESI-MS spectrum exhibited a molecular ion at *m/z* 345 $[M+H]^+$, so the molecular formula of **1** was determined as $C_{17}H_{12}O_8$. From NMR and MS data, compound **1** was determined as 3,3',4'-tri-*O*-methyl ellagic acid [10].

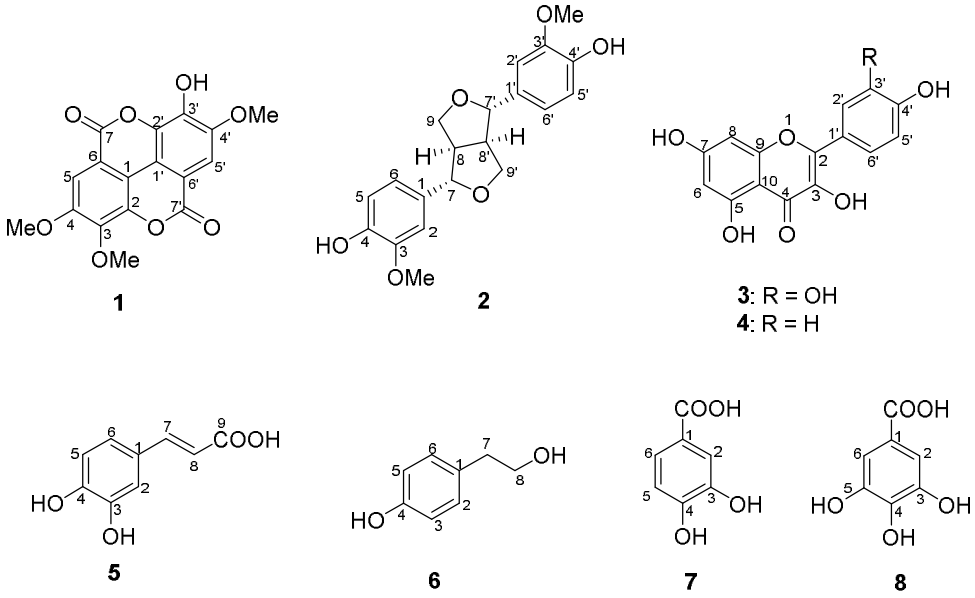

Fig. 1. Chemical structures of isolated compounds **1**–**8**

Compound **2** was obtained as a white powder. The negative ESI-MS spectrum displayed an ion *m/z* 357 $[M-H]^-$, which combined with the NMR data, suggested the molecular formula $C_{20}H_{22}O_6$. The molecular formula indicated that **1** has a symmetrical structure with the duplication of NMR signals. The ^{1}H-NMR spectrum showed the presence of 22 protons, including six protons belonging to two ABX aromatic ring systems at δ_H 6.98 (2H, d, J = 1.8 Hz, H-2, H-2'), 6.83 (2H, dd, J = 8.4, 1.8 Hz, H-6, H-6'), and 6.78 (2H, d, J = 8.0 Hz, H-5, H-5'); two oxymethine protons at δ_H 4.67 (2H, d, J = 4.2 Hz, H-7, H-7'); four protons of two oxymethylene groups at δ_H 4.20 (2H, m, Ha-9, Ha-9') and 3.80 (2H, m, Hb-9, Hb-9'); two methoxy groups at δ_H 3.84 (6H, s, 3-OMe, 3'-OMe); and two methine protons at δ_H 3.08 (2H, m, H-8, H-8'). The ^{13}C-NMR spectrum exhibited 10 signals for 20 carbons, including 12 aromatic carbon signals at δ_C 133.8 (C-1, C-1'), 111.0 (C-2, C-2'), 149.1 (C-3, C-3'), 147.3 (C-4, C-4'), 116.1 (C-5, C-5'), and 120.1 (C-6, C-6'); two oxymethine carbons at δ_C 87.5 (C-7, C-7'); two oxymethylene carbons at δ_C 72.6 (C-9, C-9'); two methoxy carbons at δ_C 56.5 (3-OMe, 3'-OMe); and two methine carbons at δ_C 55.3 (C-8, C-8'). Based on the above spectral data and comparison with the reported literature [11], **2** was identified as pinoresinol.

Table 1. ^{1}H- and ^{13}C-NMR data of compounds **1** and **2**

Pos.	1		δ_C[a,d] [10]	Pos.	2		δ_C[e,c] [11]
	δ_H[a,b] mult. (*J* in Hz)	δ_C[a,c]			δ_H[c,b], mult. (*J* in Hz)	δ_C[e,c]	
1		111.0	111.2	1, 1'		133.8	133.8
2		141.0	140.9	2, 2'	6.98, d (1.8)	111.0	111.9
3		140.3	140.2	3, 3'		149.1	149.1
4		152.9	152.6	4, 4'		147.3	147.2
5	7.49, s	111.8	111.6	5, 5'	6.78, d (8.4)	116.1	116.1
6		112.5	112.5	6, 6'	6.83, dd (8.4, 1.8)	120.1	120.0
7		158.4	158.3	7, 7'	4.73, d, (4.2)	87.5	87.4
1'		111.8	111.9	8, 8'	3.16, m	55.3	55.3
2'		141.4	141.5	9, 9'	4.25, m/3.85, m	72.6	72.6
3'		140.8	140.8				

Pos.	1		δ_C[a,d] [10]	Pos.	2		δ_C[e,c] [11]
	δ_H[a,b] mult. (*J* in Hz)	δ_C[a,c]			δ_H[e,b], mult. (*J* in Hz)	δ_C[e,c]	
4'		153.8	153.8	3-OMe	3.87, s	56.5	56.4
5'	7.57, s	107.5	107.5				
6'		113.4	113.4				
7'		158.7	158.4				
3-OMe	4.05, s,	61.0	61.0				
3'-OMe	4.03, s	61.3	61.3				
4'-OMe	3.98, s	56.7	56.7				

[a]: *DMSO-d_6*; [b]: *600 MHz*, [c]: *125 MHz*; [d]: *100 MHz*; [e]: *CD_3OD*;

Compound **3** was isolated as a pale yellow solid. The ^{1}H-NMR spectrum displayed two *meta*-coupled aromatic proton signals at δ_H 6.41 (1H, d, *J* = 2.4 Hz, H-8) and 6.19 (1H, d, *J* = 2.4 Hz, H-6); 3 signals corresponding to an ABX spin system of an aromatic ring at δ_H 7.68 (1H, d, *J* = 2.4 Hz, H-2'), 7.55 (1H, dd, *J* = 2.4, 8.4 Hz, H-6'), and 6.89 (1H, d, *J* = 8.4 Hz, H-5'). The ^{13}C-NMR spectrum showed 15 carbon signals of a flavonoid skeleton, including a carbonyl carbon at δ_C 175.4 (C-4), oxygenated aromatic carbons at δ_C 164.1 (C-7), 160.7 (C-5), 156.2 (C-9), 147.7 (C-4'), 146.8 (C-2), 145.1 (C-3'), and 135.7 (C-3), methine carbons at δ_C 120.0 (C-6'), 115.6(C-5'), 115.0 (C-2'), 98.2 (C-6), and 93.4 (C-8), and non-protonated aromatic carbons at δ_C 121.9 (C-1') and 102.9 (C-10). The ESI-MS of compound **3** displayed a protonated peak at *m/z* 303.1 $[M+H]^+$, in combination with the NMR data, suggested the molecular formula of **3** as $C_{15}H_{10}O_7$. Compound **3** was identified as quercetin based on comparison of its spectral data with previous literature [12].

Compound **4** was obtained as a pale yellow solid. The ^{1}H-NMR spectrum of **4** also showed characteristic signals of a flavonoid, with two *meta*-aromatic proton signals at δ_H 6.40 (1H, d, *J* = 2.0 Hz, H-8) and 6.20 (1H, d, *J* = 2.0 Hz, H-6); and signals of a 1,4-disubstituted benzene ring at δ_H 8.01 (2H, d, *J* = 8.5 Hz, H-2', H-6') and 6.92 (2H, d, *J* = 8.5 Hz, H-3', H-5'). The ^{13}C-NMR spectrum of **4** showed signals for 15 carbons of a flavonoid skeleton. The ESI-MS of **4** exhibited an ion at *m/z* 287.1 $[M+H]^+$, which suggested the molecular formula $C_{15}H_{10}O_6$. Compound **4** was determined as kaempferol (Fig. 1) based on the agreement with the reported spectral data [13].

Table 2. ^{1}H- and ^{13}C-NMR data of compounds **3** and **4**

Pos.	3		δ_C[a,c] [12]	4		δ_C [13]
	δ_H[a,b], mult. (*J* in Hz)	δ_C[a,c]		δ_H[d,e], mult. (*J* in Hz)	δ_C[d,c]	
2	-	146.8	146.8	-	148.0	148.0
3	-	135.7	135.6	-	137.1	137.1
4	-	175.8	175.9	-	177.3	177.3
5	-	160.7	160.8	-	162.5	162.5
6	6.19, d (2.4)	98.2	98.3	6.20, d (2.0)	99.3	99.3
7	-	164.1	164.2		165.5	165.6
8	6.41, d (2.4)	93.4	93.4	6.40, d (2.0)	94.5	94.4
9	-	156.2	156.2	-	158.2	158.2
10	-	102.9	102.0	-	104.5	104.5
1'	-	121.9	122.1	-	123.7	123.7
2'	7.68, d (2.4)	115.0	115.0	8.01, d (8.5)	130.7	130.7
3'	-	145.1	145.1	6.92, d (8.5)	116.3	116.3
4'	-	147.7	147.8	-	160.5	160.5
5'	6.89, d (8.4)	115.6	115.7	6.92, d (8.5)	116.3	116.3
6'	7.55, dd (2.4, 8.4)	120.0	120.0	8.01, d (8.5)	130.7	130.7
5-OH	12.48, s					

[a]: DMSO-d_6; [b]: 600 MHz, [c]: 125 MHz; [d]: CD_3OD; [e]: 500 MHz.

Compound **5** was obtained as a white solid. The ^{1}H-NMR spectrum showed signals of an A_2B_2 system of an aromatic ring at δ_H 6.78 (2H, d, *J* = 8.4 Hz, H-3, H-5), 7.08 (2H, d, *J* = 8.4 Hz, H-2, H-6), and two methylene groups at δ_H 2.80 (2H, t, *J* = 6.6 Hz, H-7), 3.82 (2H, t, *J* = 6.6 Hz, H-8).

The ^{13}C-NMR spectrum showed signals for 8 carbons, with 6 signals for the aromatic ring at δ_C 131.0 (C-1), 130.9 (C-2, C-6), 116.1 (C-3, C-5), and 156.78 (C-4), an oxymethylene group at δ_C 64.8 (C-8), and a methylene group at δ_C 39.4 (C-7). Compound **5** was identified as tyrosol based on comparison of its spectral data with the reference literature [14].

Compound **6** was isolated as a pale yellow powder. The ^{1}H-NMR spectrum of **6** showed the presence of an ABX system with three protons at δ_H 7.05 (1H, d, J = 2.0 Hz, H-2), 6.94 (1H, dd, J = 2.0, 8.5 Hz, H-6), and 6.79 (1H, d, J = 8.5 Hz, H-5). In addition, there were two *trans* olefinic protons at δ_H 7.53 (1H, d, J = 15.5 Hz, H-7) and 6.25 (1H, d, J = 15.5 Hz, H-8). The ^{13}C-NMR spectrum of **6** showed signals for 9 carbons, with 6 signals for an aromatic ring at δ_C 149.4 (C-3), 146.8 (C-4), 127.9 (C-1), 122.8 (C-6), 115.9 (C-2), and 115.1 (C-5), two olefinic carbons at δ_C 146.8 (C-7) and 116.5 (C-8), and one carboxylic acid group at δ_C 171.3 (COOH). Based on the above spectral data and comparison with reference literature, **6** was identified as caffeic acid [15].

Compounds **7** and **8** were identified as protocatechuic acid [16] and gallic acid [9], respectively, based on the comparison of NMR data with those in the previous literature.

Table 3. ^{1}H- and ^{13}C-NMR data of compounds **5** and **6**

Pos.	5		δ_C[a,c] [14]	6		δ_C[a,c] [15]
	δ_H[a,b], mult. (J in Hz)	δ_C[a,c]		δ_H[a,d], mult. (J in Hz)	δ_C[a,c]	
1		131.0	131.0		127.9	126.4
2	7.08, d (8.4)	130.9	130.9	7.05, d (2.0)	115.9	113.7
3	6.78, d (8.4)	116.1	116.1		149.4	145.6
4		156.8	156.8		146.8	145.4
5	6.78, d (8.4)	116.1	116.1	6.79, d (8.5)	115.1	115.1
6	7.08, d (8.4)	130.9	130.9	6.94, dd (2.0, 8.5)	122.8	121.4
7	2.80, t (6.6)	39.4	39.4	7.53, d (15.5)	146.8	148.1
8	3.82, t (6.6)	64.8	64.6	6.25, d (15.5)	116.5	114.8
9				-	171.3	169.6

[a]: CD_3OD; [b]: 600 MHz, [c]: 125 MHz, [d]: 500 MHz.

Our work represents the first isolation study of *C. flava* flowers. Several ellagic acids have already been found from *Camellia sp* [1], but 3,3',4'-tri-*O*-methyl ellagic acid (**1**) was identified in the genus *Camellia* for the first time. Lignan pinoresinol was isolated from *C. olifera* [17]. Flavonoids quercetin (**3**), kaempferol (**4**), and phenolic acids **5**–**7** were identified from flowers and leaves of many *Camellia* species like *C. chrysantha* [18], *C. flava* [9], *C. olifera* [19], *C. sinensis* [20], and *C. japonica* [21]. Tyrosol (**5**) was reported from *C. olifera* [22] and *C. japonica* [23]. Our chemical study suggested that *C. flava* possibly has a close chemotaxonomic relationship with *C. olifera* and *C. japonica.* These phenolic constituents displayed various pharmacological activities such as antioxidant, anti-diabetic, and neuroprotective activities [19],[21],[23],[24],[25].

4. Conclusions

Eight phenolic constituents, including 3,3',4'-tri-*O*-methyl ellagic acid (**1**), pinoresinol (**2**), quercetin (**3**), kaempferol (**4**), tyrosol (**5**), caffeic acid (**6**), protocatechuic acid (**7**), and gallic acid (**8**), were isolated and characterized from the ethyl acetate extract of *Camellia flava* flowers. Compounds **2**, **3**, and **5**–**7** were identified for the first time from this species, and compound **1** was first reported from the *Camellia* genus.

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