Phenolic Compounds and Carotenoids from the Leaves of *Gymnosporia chevalieri* Tard.

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Abstract

Background: The *Gymnosporia* genus holds substantial potential for bioactive compound discovery, yet its phytochemical composition remains underexplored relative to its botanical diversity. In Vietnam, aside from preliminary studies on *G. stylosa*, systematic research on *Gymnosporia* species is notably lacking. This study provides the understanding of the chemical constituents of *G. chevalieri*, establishing a basis for future research on its potential applications and bioactive properties. **Materials and methods:** The leaves of *G. chevalieri* were macerated in methanol to produce a crude extract, which was further fractionated via liquid-liquid partitioning using *n*-hexane and ethyl acetate as solvents. Compounds were then isolated and purified using various chromatographic techniques. Structural elucidation was achieved through 1D- and 2D-NMR and HR-ESI-MS analysis, supported by comparison with previously reported spectroscopic data. **Results & Conclusion:** Seven phenolic compounds and two carotenoids were isolated from *G. chevalieri* leaves. These were identified as syringaldehyde (**1**), 3,7-dihydroxy-6-methoxy-2,7-dimethyldibenzofuran (**3**), atalantoflavone (**4**), lutein (**6**), lutein 3'-methyl ether (**7**), and two mixtures: 4-hydroxybenzaldehyde (**2a**) with 4-hydroxy-3-methoxybenzaldehyde (**2b**), and breynioside B (**5a**) with 6'-*O*-vanilloylarbutin (**5b**). Notably, compounds **2**-**7** were isolated from the *Gymnosporia* genus for the first time.

Keywords: *Gymnosporia chevalieri*, *phenolic compounds*, *carotenoid.*

1. INTRODUCTION

The genus *Gymnosporia* (Celastraceae family) comprises approximately 116 species worldwide [1]. In Vietnam, the genus *Gymnosporia* includes 8 recorded species, among which *G. chevalieri* is an endemic species located in the Binh Tri Thien area [2]. In the search for potential bioactive compounds from the genus *Gymnosporia*, phenolics were found alongside other active groups [3, 4]. This article reports, for the first time, the extraction, isolation, and structural identification of seven phenolic compounds and two carotenoids from the leaves of *G. chevalieri* (Figure 1).

Figure 1. The leaves and flowers of *Gymnosporia chevalieri* Tard.

Phenolics and carotenoids are two major groups of plant compounds celebrated for their significant health benefits, especially for their roles in antioxidant defense, reducing inflammation, and

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protecting cells [5, 6]. The presence of phenolic compounds and carotenoids in *G. chevalieri* is not only significant as a phytochemical database but also as a basis for orienting studies on the antioxidant and anti-inflammatory activities of this species.

2. MATERIALS AND METHODS

Materials

The species *Gymnosporia chevalieri* was collected in October 2023 from Đakrong District, Quang Tri Province. Dr. Anh Tuan Le from the Mien Trung Institute for Scientific Research (Vietnam National Museum of Nature, VAST) identified the species. A voucher specimen (GC-01) has been stored at the Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Vietnam.

Methods

The leaves of *G. chevalieri* were cleaned, air-dried, and ground into powder. The powdered material was extracted by maceration with MeOH (10 L x 3 times), and the solvent was removed under reduced pressure to yield a crude extract. The total extract undergoes sequential liquid-liquid distribution with solvents of increasing polarity, after which the solvents are evaporated to yield the corresponding fractions.

The isolation process involves a combination of thin-layer chromatography (TLC) and column chromatography (CC). TLC was conducted on pre-coated DC-Alufolien 60 F_{254} and RP18 F_{254} plates (Merck, Germany). Compounds were detected by UV light at wavelengths of 254 and 365 nm or by spraying the plates with a 10% H_2SO_4 solution and heating them until color developed. Column chromatography was performed using different stationary phases, including normal silica gel (40–63 µm, Merck, Germany), reverse-phase RP-18 (30–50 µm, Fuji Silysia Chemical, Japan), sephadex LH-20, and MCI gel (Sigma-Aldrich, USA).

The chemical structures were elucidated using 1D-, 2D-NMR, HR-ESI-MS spectra, in conjunction with comparison to reference spectral data. NMR spectra were recorded on a Bruker Avance Neo 600 Spectrometer (Bruker, Massachusetts, USA). Tetramethylsilane was employed as the internal standard. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) data were acquired on SCIEX X500 QTOF spectrometers (SCIEX, Massachusetts, USA).

3. RESULTS

3.1. Extraction and isolation

A total crude extract (GCM, 930 g) was achieved by extraction of dry leaves powder (4 kg) of *G. chevalieri.* After performing liquid-liquid distribution extraction with *n*-hexane and EtOAc (each, 4 L x 3 times). Three fractions were abtained including *n*-hexane (GCH; 201.8 g), EtOAc (GCE; 97.9 g), and aqueous extract (GCW; 525 g).

The GCH extract was subjected to silica gel column using a gradient elution of *n*-hexane/acetone (100:0, 80:1, 40:1, 20:1, 10:1, 5:1, 2:1, 1:1, and 0:100, v/v) to afford six fractions, GCH1-GCH6. Fraction GCH5 (30.3 g) was separated by silica gel column, eluting with a gradient of *n*-hexane/acetone (10:1, 5:1, 2:1, 1:1, v/v), yielding four fractions: GCH5.1–GCH5.4. Fraction GCH5.3 (7.67 g) was separated using a RP-18 column, eluted with acetone/water (5:1, v/v) to obtain six fractions: GCH5.3.1–GCH5.3.6.

Fraction GCH5.3.1 (770.8 mg) was carried out over the sephadex eluted with CH_2Cl_2/MeOH (1:1, v/v) yielded four fractions: GCH5.3.1.1–GCH5.3.1.4. Fraction GCH5.3.1.3 (47.8 mg) was further separated using a RP-18 column, eluted successively with MeOH/water (3:2, v/v) and acetone/water (2:1, v/v), yielding five fractions: GCH5.3.1.3.1–GCH5.3.1.3.5. Fraction GCH5.3.1.3.1 (9.4 mg) was separated using a sephadex column, eluted with CH_2Cl_2/MeOH (1:1, v/v), yielding two fractions: GCH5.3.1.3.1.1–GCH5.3.1.3.1.2. Fraction GCH5.3.1.3.1.1 (4.8 mg) was purified using a sephadex column, eluted with MeOH 100% to give compound **1** (3 mg). Fraction GCH5.3.1.3.1.2 (4.7 mg) was purified using a silica gel column, eluted with *n*-hexane/acetone (5:1, v/v), yielding compound **2** (3 mg). Fraction GCH5.3.1.3.4 (4.8 mg) was crystallized, resulting in compound **4** (1.3 mg).

Fraction GCH5.3.1.4 (9.8 mg) underwent purification using a RP-18 column with acetone/water (2:1, v/v), followed by further purification on a sephadex column using MeOH 100%, yielding compound **3** (3 mg).

Fraction GCH5.3.4 (630 mg) was separated using a silica gel column, eluted with CH_2Cl_2/acetone (30:1, v/v) to give three fractions: GCH5.3.4.1–GCH5.3.4.3. Fraction GCH5.3.4.3 (230 mg) was further separated using a silica gel column, eluted with an *n*-hexane/CH_2Cl_2/acetone (1:1:0.1, v/v), yielding three fractions: GCH5.3.4.3.1–GCH5.3.4.3.3. Fraction GCH5.3.4.3.1 (76 mg) was carried out over a RP-18 column by eluted with acetone/water (5:1, v/v) to afford four fractions: GCH5.3.4.3.1.1–GCH5.3.4.3.1.4. Compound **6** (11.7 mg) was obtained from sub-fraction GCH5.3.4.3.1.4 (21 mg).

Fraction GCH5.1 (3.6 g) was subjected to silica gel column with a gradient of *n*-hexane/EtOAc (5:1,

3:1, v/v), giving eight fractions: GCH5.1.1–GCH5.1.8. Fraction GCH5.1.6 (650 mg) was separated by MCI gel, eluting with a gradient of MeOH/water (100:0, 8:2, and 6:4, v/v), yielding four fractions: GCH5.1.6.1–GCH5.1.6.4. Fraction GCH5.1.6.4 (116.3 mg) was separated using a sephadex column with CH_2Cl_2/MeOH (1:1, v/v), followed by a RP-18 column with acetone/MeOH/water (1:1:0.1, v/v), yielding two fractions: GCH5.1.6.4.1 and GCH5.1.6.4.2. Fraction GCH5.1.6.4.2 (83.9 mg) was purified using a silica gel column, eluted with an *n*-hexane/acetone (6:1, v/v), yielding compound **7** (8.4 mg).

The EtOAc extract (GCE; 97.9 g) was loaded on a silica gel column eluted with a stepwise solvent gradient of CH_2Cl_2/MeOH (20:1, 10:1, 5:1, 2:1, 1:2, 0:100, v/v) to obtain six fractions: GCE1–GCE6. Fraction GCE3 (38 g) was separated on a RP-18 column by eluted with MeOH/water (1:2, v/v) to yield four sub-fractions: GCE3.1–GCE3.4. Fraction GCE3.1 (6.33 g) was separated on the silica gel column and eluted sequentially with CH_2Cl_2/EtOAc/MeOH (3:1:0.4, v/v) and CH_2Cl_2/EtOAc/MeOH (3:1:0.6, v/v) to obtain six fractions: GCE3.1.1–GCE3.1.6. Fraction GCE3.1.3 (62.0 mg) was carried out over the sephadex eluted with MeOH 100% to afford two fractions: GCE3.1.3.1–GCE3.1.3.2. Fraction GCE3.1.3.1 (12.0 mg) was purified sequentially using a RP-18 column with MeOH/water (2:3, v/v), followed by a sephadex column with MeOH 100%, yielding compound **5** (4.9 mg).

3.2. Structural determination of isolates

Compound **1** was isolated as a white powder. The ^{1}H-NMR spectrum showed a characteristic resonance signal for a formyl group at δ_H 9.82 (s), two aromatic protons of a 1,3,4,5-tetrasubstituted benzene ring at δ_H 7.16 (2H, s), and two methoxy groups at δ_H 3.98 (6H, s). The presence of the formyl and methoxy groups was further confirmed by the ^{13}C-NMR spectrum, which showed characteristic signals at δ_C 190.7 and 56.5, respectively. Signals at δ_C 106.8 (C), 128.5 (2CH), 147.4 (2C), and 141.3 (C) were consistent with a substituted benzene ring. Comparison with reference data [7] identified compound 1 as syringaldehyde, also known as 4-hydroxy-3,5-dimethoxybenzaldehyde (Figure 2).

The ^{1}H-NMR spectrum of **2** showed resonance signals for two formyl groups at δ_H 9.87, 9.83 (each, 1H, s); four aromatic protons of a 1,4-disubstituted benzene ring at δ_H 7.81, 6.96 (each, 2H, d, J = 8.6 Hz); three aromatic protons of a 1,3,4-trisubstituted benzene ring at δ_H 7.42 (1H, d, J = 1.9 Hz), 7.05 (1H, d, J = 8.5 Hz), and 7.43 (1H, dd, J = 8.5, 1.9 Hz); and one methoxy group at δ_H 3.97 (3H, s). The ^{13}C-NMR spectrum displayed 15 carbon signals, including two carbonyl carbons (δ_C 191.0, 190.8); three oxygenated sp^2 carbons (δ_C 161.3, 151.7, 147.2); nine sp^2 carbons [δ_C 132.4 (2CH), 130.1 (C), 129.9 (C), 127.6 (CH), 115.9 (2CH), 114.4 (CH), 108.8 (CH)]; and one methoxy carbon (δ_C 56.2) (Table 1). Comparison with reference data [8], [9] led to the identification of compound **2** as a mixture of 4-hydroxybenzaldehyde (**2a**) and 4-hydroxy-3-methoxybenzaldehyde (**2b**) (also known as vanillin).

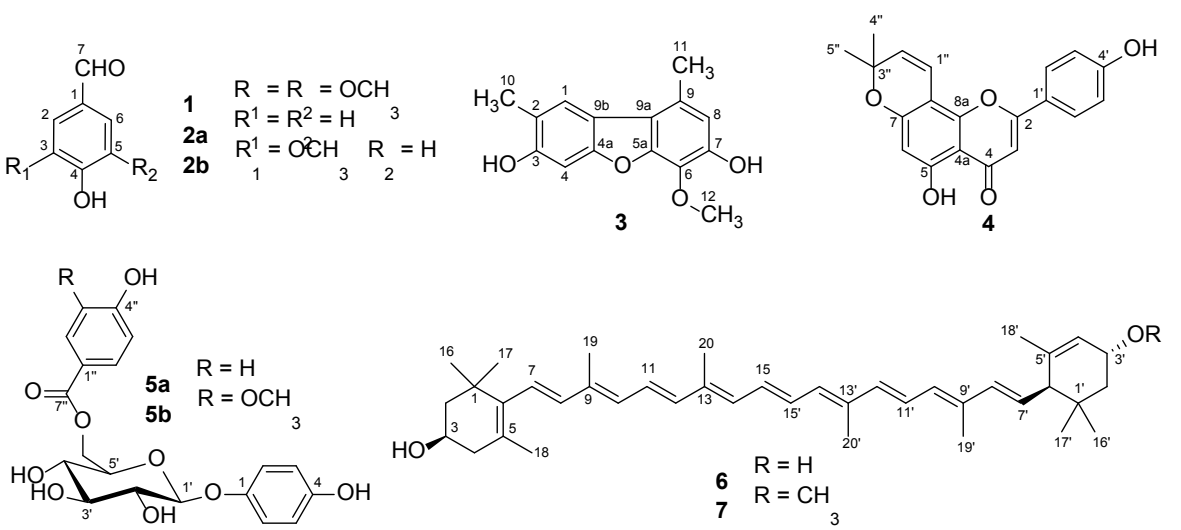

Figure 2. Structures of phenolic compounds (**1**-**5**) and carotenoids (**6**, **7**) isolated from *Gymnosporia chevalieri*

Compound **3** was isolated as a white powder. The ^{1}H-NMR spectrum displayed characteristic resonance signals of three aromatic protons at δ_H 7.61, 7.00, and 6.71 (each, 1H, s); one methoxy group at δ_H 4.19 (3H, s); and two methyl groups at δ_H 2.64 and 2.38 (each, 3H, s). The ^{13}C-NMR and HSQC spectra indicated that molecule **3** consists of 15 carbons, categorized as three methyls (δ_C 61.2, 19.4, 16.2), three methines (δ_C 122.6, 111.8, 98.3), and nine quaternary carbons. Signals at δ_C 155.8, 152.3, 146.9, and 145.9 were assigned to oxygenated sp^2 carbons. The δ_C/δ_H 61.2/4.19 signal confirmed the presence of a methoxy group attached to the aromatic ring (Table 2). These data suggest that compound **3** is a dibenzofuran derivative.

The HMBC correlations between H-1 (δ_H 7.61)/H-4 (δ_H 7.61) and C-2 (δ_C 119.2)/C-3 (δ_C 152.3)/C-4a (δ_C 155.8)/ C-9b (δ_C 117.6) confirm the presence of a 1,2,4,5-tetrasubstituted benzene ring (Figure 3). The remaining singlet at δ_H 6.71 was attributed to a 1,2,3,4,5-pentasubstituted benzene ring. Notably, strong HMBC correlations between H_3-10 (δ_H 2.38) and C-1 (δ_C 122.6)/C-2/C-3; between H_3-11 (δ_H 2.64) and C-8 (δ_C 111.8)/C-9 (δ_C 122.6)/C-9a (δ_C 117.6); and between H_3-12 (δ_H 4.19) and C-6 (δ_C 129.8) located two methyl groups and the methoxy group at C-2, C-8, and C-6, respectively, on the dibenzofuran skeleton. These analyses identified the structure of compound 3 as 3,7-dihydroxy-6-methoxy-2,7-dimethyldibenzofuran [10].

Table 1. NMR data of **1**, **2** and reference compounds in $CDCl_3$ [δ (ppm), J (Hz)]

C	1			2a			2b		
	$\delta_C^{\#}$	δ_C^{a}	δ_H^{b}	δ_C^{*}	δ_C^{a}	δ_H^{b}	$\delta_C^{\$}$	δ_C^{a}	δ_H^{b}
1	106.9	106.8	–	128.7	129.9	–	130.1	130.1	–
2	128.6	128.5	7.16s	132.6	132.4	7.81 d (8.6)	108.9	108.8	7.42 d (1.9)
3	147.5	147.4	–	116.1	115.9	6.96 d (8.6)	147.3	147.2	–
4	141.1	141.3	–	161.1	161.3	–	151.8	151.7	–
5	147.5	147.4	–	116.1	115.9	6.96 d (8.6)	114.5	114.4	7.05 d (8.5)
6	128.6	128.5	7.16s	132.6	132.4	7.81 d (8.6)	127.7	127.6	7.43 dd (8.5, 1.9)
7	190.6	190.7	9.82s	191.1	191.0	9.83 s	191.0	190.8	9.87 s
OCH_3	–	56.5	3.98s	–	–	–	56.3	56.2	3.97 s
OH	–	–	5.30 s	–	–	–	–	–	6.19 s

$^{\#, *, \$}\delta_C$ values of syringaldehyde, 4-hydroxybenzaldehyde, vanillin, respectively [7], [8], [9], ^{a}150 MHz, ^{b}600 MHz

Compound **4** was isolated as a yellow powder. The ^{1}H-NMR spectrum showed a characteristic resonance signal for a hydroxyl proton at δ_H 12.85 (s); four aromatic protons of a 1,4-disubstituted benzene ring at δ_H 7.79, 6.97 (each 2H, d, J = 8.4 Hz); four olefinic protons at δ_H 6.78, 5.61 (each 1H, d, J = 10.0 Hz), 6.56, 6.28 (each 1H, s). Additionally, two equivalent methyl groups were observed in the high-field region at δ_H 1.49 (6H, s) (Table 2).

The ^{13}C-NMR spectrum showed resonances mainly in the downfield region (δ_C > 100) except for an oxygenated sp^3 carbon at δ_C 78.0 and methyl carbons at δ_C 28.2. A carbonyl group appeared at δ_C 182.6, and signals between δ_C 150-165 indicated oxygenated sp^2 carbons. Furthermore, HSQC data indicated eight sp^2 CH groups and two CH_3 groups. This evidence suggests that compound **4** is a prenylated flavone.

Key HMBC correlations were observed between H-3 (δ_H 6.56) and C-2 (δ_C 163.5)/C-4 (δ_C 182.6)/C-4a (δ_C 105.2)/ C-1' (δ_C 124.0); between H-2',6' (δ_H 7.79) and C-2/C-4' (δ_C159.1); and between 5-OH (δ_H 12.85) and C-4a/C-5 (δ_C 161.8)/C-6 (δ_C 100.3), confirming the flavone skeleton of compound **4**. Additionally, correlations between the two methyl groups (δ_H 1.49) and C-2" (δ_C 127.5)/C-3" (δ_C 78.0); between H-1" (δ_H 6.78) and C-7 (δ_C 159.7)/C-3"; and between H-2" (δ_H 5.61) and C-8 (δ_C 101.3) enabled the construction of a 2,2-dimethyl-2*H*-pyran ring. The attachment of this ring to the flavone moiety at C-7/C-8 was confirmed by the correlations H-6 (δ_H 6.28) with C-7/C-8, H-1" with C-7/C-8a, and

H-2" with C-8. From these analyses, compound **4** is proposed to be 2-(4-hydroxyphenyl)-5-hydroxy-8,8-dimethyl-4*H*,8*H*-pyrano[2,3-f]chromen-4-one, also known as atalantoflavone [11].

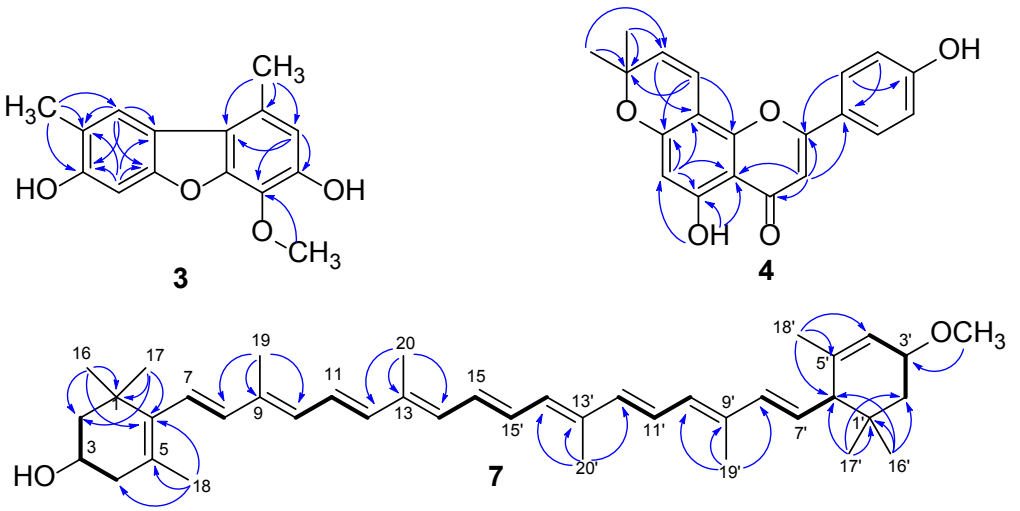

Figure 3. Selected HMBC (arrow), COSY (bold line) correlations of **3**, **4** and **7**

The ^{1}H-NMR spectrum of **5** showed characteristic resonance signals for three 1,4-disubstituted benzene rings with 12 protons at δ_H 7.92, 6.95, 6.94, 6.88, 6.62, and 6.60 (each 2H, d, *J* = 8.8 Hz), as well as one 1,3,4-trisubstituted ring with three protons at δ_H 7.61 (1H, dd, *J* = 8.2, 2.0 Hz), 7.58 (1H, d, *J* = 2.0 Hz), and 6.90 (1H, d, *J* = 8.2 Hz). Resonances corresponding to two β-anomeric protons for sugar units were observed at δ_H 4.76 and 4.75 (each 1H, d, *J* = 7.5 Hz), and a signal at δ_H 3.89 (3H, s) was assigned to a methoxy group (Table 3).

The ^{13}C-NMR and HSQC spectra showed two carboxyl carbons (δ_C 167.95, 167.91), fifteen sp^2 methine carbons [δ_C 132.9 (2C), 119.6 (2C), 119.6 (2C), 116.6 (4C), 116.2 (2C), 125.3, 116.0, 113.8], ten sp^3 methine carbons [δ_C 103.7 (2C), 75.0 (2C), 75.6 (2C), 78.0 (2C), 72.1 (2C)], two sp^3 oxymethylene carbons (δ_C 65.2, 65.1), and one methoxy carbon (δ_C 56.5).

Overall, the ^{1}H- and ^{13}C-NMR signals appeared as pairs with an approximate ratio of 1.5:1, indicating that compound **5** is a mixture of two phenolic glycosides. The presence of two carboxyl carbons (δ_C 168.0, 167.9) along with the downfield shift of oxymethylene carbons suggested esterification at the C-6' of the sugar moiety. Comparison with reference data allowed the identification of compound **5** as a mixture of breynioside B (**5a**) [12] and 6'-*O*-vanilloylarbutin (**5b**) [13].

Table 2. NMR data of **3**, **4** (in $CDCl_3$) and reference compounds [δ (ppm), *J* (Hz)]

C	3			C	4		
	δ_C [#]	δ_C [a]	δ_H [b]		δ_C [*]	δ_C [a]	δ_H [b]
1	123.4	122.6	7.61 s	2	164.9	163.5	-
2	121.6	119.2	-	3	104.4	104.4	6.56 s
3	155.4	152.3	-	4	183.3	182.6	-
4	98.5	98.3	7.00 s	5	162.9	161.8	-
4a	157.3	155.8	-	6	100.7	100.3	6.28 s
5a	149.6	146.9	-	7	160	159.7	-
6	132.2	129.8	-	8	102.2	101.3	-
7	148.4	145.9	-	8a	152.6	152.1	-
8	114	111.8	6.71 s	4a	106.1	105.2	-

9	127.6	126.6	-	1’	122.6	124.0	-
9a	118.4	117.6	-	2’	129.4	128.2	7.79 d (8.4)
9b	118.3	118.3	-	3’	117.4	116.2	6.97 d (8.4)
10	16.7	16.2	2.38 s	4’	163.4	159.1	
11	19.6	19.4	2.64 s	5’	117.4	116.2	6.97 d (8.4)
12	61.5	61.1	4.19 s	6’	129.4	128.2	7.79 d (8.4)
				1’’	115.7	114.9	6.78 d (10.0)
				2’’	128.2	127.5	5.61 d (10.0)
				3’’	78.7	78.0	-
				4’’, 5’’	28.5	28.2	1.49 s
				OH	-	-	12.85 s

[#]δ_C values of 3,7-dihydroxy-6-methoxy-2,7-dimethyldibenzofuran in CD_3OD [10], [*]δ_C values of atalantoflavone in C_5D_5N [11], [a]150 MHz, [b]600 MHz.

Table 3. NMR data of mixture **5a**:**5b** and reference compounds in CD_3OD [δ (ppm), J (Hz)]

C	5a			5b		
	δ_C [#]	δ_C [a]	δ_H [b]	δ_C [*]	δ_C [a]	δ_H [b]
1	152.3	152.3	-	152.1	152.3	-
2	119.6	119.6	6.95 d (8.8)	116.5	119.6[d]	6.94 d (8.8)
3	116.6	116.6	6.62 d (8.8)	119.4	116.6[d]	6.60 d (8.8)
4	153.9	153.9	-	153.6	153.9	-
5	116.6	116.6	6.62 d (8.8)	119.4	116.6	6.60 d (8.8)
6	119.6	119.6	6.95 d (8.8)	116.5	119.6	6.94 d (8.8)
1’	103.7	103.7	4.76 d (7.5)	103.4	103.7	4.75 d (7.5)
2’	74.9	75.0	3.48 m	77.8	75.0[e]	3.48 m
3’	78.2	78.0	3.49 m	74.9	78.0[e]	3.49 m
4’	72.1	72.1	3.43 m	72.0	72.1	3.43 m
5’	75.5	75.6	3.73 m	75.3	75.6	3.73 m
6’	65.1	65.2	4.71 dd (11.8, 2.2) 4.39 dd (11.8, 7.6)	65.1	65.1	4.69 dd (11.8, 2.2) 4.37 dd (11.8, 7.5)
1’’	122.2	122.5	-	122.3	122.2	-
2’’	132.9	132.9	7.92 d (8.8)	113.6	113.8	7.58 d (2.0)
3’’	116.2	116.2	6.88 d (8.8)	148.6	148.8	-
4’’	163.6	163.7	-	152.7	153.1	-
5’’	116.2	116.2	6.88 d (8.8)	115.9	116.0	6.90 d (8.2)
6’’	132.9	132.9	7.92 d (8.8)	125.1	125.3	7.61 dd (8.2, 2.0)
7’’	167.9	168.0	-	167.8	167.9	-
-	-	-	-	56.4	56.5	3.89 s

[#]δ_C values of breynioside B, [*]δ_C values of 6’-O-vanilloylarbutin [12], [13], [a]150 MHz, [b]600 MHz, [d,e]resassignment.

Compound **6** was obtained as an orange-red powder. Its ^{1}H-NMR spectrum exhibited features characteristic of a polyene, with resonances of olefinic protons at δ_H 6.63 (3H, m), 6.36 (2H, d, *J* = 14.9 Hz), 6.25 (2H, d, *J* = 9.6 Hz), 6.15 (3H, m), 6.12 (2H, m), 5.54 (1H, s), and 5.43 (1H, dd, *J* = 15.5, 9.9 Hz). Signals for two carbinol groups were observed at δ_H 4.25 (1H, s) and 4.00 (1H, m). In addition, singlet signals of ten methyl groups appeared at δ_H 1.97 (9H), 1.91, 1.73, 1.63, 1.07 (6H), 1.00, and 0.85, where signals with higher δ_H values (1.97, 1.91, 1.73, and 1.63) are typically attributed to methyl groups attached to double bonds (Table 4).

The combination of ^{13}C-NMR and HSQC spectra revealed a total of 40 carbons in this compound, including 10 CH_3, 3 CH_2, 18 CH, and 9 quaternary carbons. Signals of olefinic carbons and two oxymethine carbons appeared at δ_C 124–138 (22C) and 65.9, 65.1, respectively. The one-dimensional NMR data suggest that compound **6** is a carotenoid. Cross-referencing with literature data [14] confirmed the identity of compound **6** as lutein.

Compound **7** exhibits NMR spectral data closely resembling that of compound **6**, with the main distinction being an additional methoxy group signal at δ_C/δ_H 55.8/3.38 (3H, s). The HR-ESI-MS spectrum shows a pseudomolecular ion at *m/z* 583.4482 $[M+H]^+$ (theoretical *m/z* for $C_{41}H_{59}O_2$ is 583.4515), consistent with the molecular formula $C_{41}H_{58}O_2$. Detailed analysis of the ^{13}C-NMR spectrum revealed that the δ_C values for the C-2′, C-3′, and C-4′ positions in compound **7** differ from those in compound **6** by 4.2, 8.7, and 2.6 ppm, respectively, while values at other positions vary by less than 0.4 ppm (see Table 4). This suggests that the methoxy group is positioned at C-3′, an assignment confirmed by an HMBC correlation between the methoxy proton (δ_H 3.38) and C-3′ (δ_C 74.6). Based on this evidence, compound **7** is identified as lutein 3′-methyl ether [14].

Table 4. NMR data of **6**, **7** and reference compounds in $CDCl_3$ [δ (ppm), *J* (Hz)]

C	6			7		
	δ_C [#]	δ_C [a, b]	δ_H [a, c] (*J*, Hz)	δ_C [*]	δ_C [a, b]	δ_H [a, c] (*J*, Hz)
1	37.1	37.1	–	37.1	37.1	–
2	48.4	48.5	1.77 ddd (12.0, 3.6, 2.0); 1.48 t (12.0)	48.4	48.5	1.77 m; 1.48 t (11.9)
3	65.1	65.1	4.00 m	65.1	65.1	4.00 m
4	42.5	42.6	2.39 dd (16.7, 4.9); 2.04 dd (16.7, 9.6)	42.5	42.6	2.37 m; 2.04 m
5	126.2	126.2	–	126.2	126.2	–
6	137.8	137.7	–	137.7	137.8	–
7	125.6	125.6	6.12 m	125.6	125.6	6.12 m
8	138.5	138.5	6.12 m	138.5	138.5	6.12 m
9	135.7	135.7	–	135.7	135.7	–
10	131.3	131.3	6.15 m	131.3	131.3	6.14 m
11	124.9	125.0	6.63 m	124.9	124.9	6.63 m
12	137.6	137.6	6.36 d (14.9)	137.5	137.6	6.35 d (14.9)
13	136.5	136.5	–	136.5	136.5	–
14	132.6	132.6	6.25 d (9.6)	132.6	132.6	6.25 d (9.2)
15	130.1	130.1	6.63 m	130.0	130.1	6.62 m
16	28.7	28.7	1.07 s	28.7	28.7	1.07 s
17	30.2	30.3	1.07 s	30.2	30.3	1.07 s
18	21.6	21.6	1.73 s	21.6	21.6	1.73 s

19	12.7	12.8	1.97 s	12.8	12.8	1.97 s
20	12.8	12.8	1.97 s	12.8	12.8	1.97 s
1′	34.0	34.0	–	33.9	33.9	–
2′	44.6	44.7	1.84 dd (13.2, 5.9); 1.36 dd (13.2, 6.8)	40.4	40.4	1.76 m; 1.40 dd (13.0, 6.9)
3′	65.9	65.9	4.25 s	74.6	74.6	3.78 s
4′	124.5	124.5	5.54 s	121.8	121.9	5.60 s
5′	138.0	137.8	–	138.2	138.2	–
6′	55.0	55.0	2.41 d (9.9)	55.1	55.2	2.42 d (9.9)
7′	128.7	128.7	5.43 dd (15.5, 9.9)	129.0	129.0	5.44 dd (15.5, 10.0)
8′	138.5	138.0	6.15 m	137.6	137.7	6.14 m
9′	135.1	135.1	–	135.1	135.1	–
10′	130.8	130.8	6.15 m	130.7	130.7	6.13 m
11′	124.8	124.8	6.63 m	124.8	124.9	6.61 m
12′	137.5	137.6	6.36 d (14.9)	137.5	137.5	6.37 d (14.9)
13′	136.4	136.4	–	136.4	136.4	–
14′	132.6	132.6	6.25 d (9.6)	132.5	132.6	6.25 d (9.2)
15′	130.0	130.1	6.63 m	130.0	130.1	6.62 m
16′	24.3	24.3	0.85 s	24.2	24.3	0.84 s
17′	29.5	29.5	1.00 s	29.4	29.5	0.97 s
18′	22.8	22.9	1.63 s	23.0	22.9	1.63 s
19′	13.1	13.1	1.91 s	13.1	13.1	1.91 s
20′	12.8	12.8	1.97 s	12.8	12.8	1.97 s
OCH_3	–	–	–	55.8	55.8	3.38 s

*#, *δ_C lutein, lutein 3′-methyl ether, respectively [14], [a]150 MHz, [b]600 MHz.*

4. DISCUSSION

Among the isolates, 3,7-dihydroxy-6-methoxy-2,7-dimethyldibenzofuran (**3**), atalantoflavone (**4**), lutein (**6**), lutein 3′-methyl ether (**7**), and two mixtures: 4-hydroxybenzaldehyde (**2a**) with 4-hydroxy-3-methoxybenzaldehyde (**2b**), and breynioside B (**5a**) with 6′-*O*-vanilloylarbutin (**5b**) were isolated from the *Gymnosporia* genus for the first time. Meanwhile, syringaldehyde (**1**) has been previously identified in *G. stylosa* [3].

Syringaldehyde (**1**) is a natural phenolic compound that emerges with multiple bioactivities, such as anti-inflammation, anti-oxidation, and anti-diabetic effects have been reported [15]. Atalantoflavone (**4**), a flavone isolated from *Erythrina sigmoidea* and the root bark of *Citrus limonia*, exhibits inhibitory effects against *P. gingivalis* and shows cytotoxic activity against multidrug-resistant cancer cell lines [16], [17]. The potential benefits of lutein (**6**) have been evaluated for neurological disorders, eye diseases, cardiac complications, microbial infections, skin irritation, bone decay, and more, with recent studies highlighting the effectiveness of lutein nanoformulations in improving eye disorders [18]. Compound 4-hydroxybenzaldehyde (**2a**) demonstrated a protective effect by decreasing lipid peroxidation levels and exhibited a potent inhibitory effect on GABA-T activity in untreated rat brain, surpassing the efficacy of valproic acid [19]. Compound vanillin (**2b**), with its antimicrobial, antioxidant, antimutagenic, hypolipidemic, anti-sickling, and anti-inflammatory properties, is widely used in pharmaceuticals such as dopamine, papaverine, methyldopa, and L-DOPA, and holds

promise in countering antimicrobial resistance [20]. Breynioside B (**5a**) isolated from *Breynia vitis-idaea* (Burm.f.) C. E. C. exhibits strong antioxidant activity [21].

5. CONCLUSION

A phytochemical investigation of *G. chevalieri* leaves led to the identification of nine secondary metabolites, including seven phenolic compounds (**1**, **2a**, **2b**, **3**, **4**, **5a**, **5b**) and two carotenoids (**6**, **7**). Through spectroscopic analysis (1D-, 2D-NMR, HR-ESI-MS) and comparison with previously reported data, these compounds were identified as syringaldehyde (**1**), 3,7-dihydroxy-6-methoxy-2,7-dimethyldibenzofuran (**3**), atalantoflavone (**4**), lutein (**6**), lutein 3′-methyl ether (**7**), and two mixtures: 4-hydroxybenzaldehyde (**2a**) with 4-hydroxy-3-methoxybenzaldehyde (**2b**), and breynioside B (**5a**) with 6′-O-vanilloylarbutin (**5b**). Compounds **2**–**7** were isolated for the first time from the *Gymnosporia* genus. These research findings deepen our understanding of the chemical composition of *G. chevalieri* and expand knowledge of the *Gymnosporia* genus as a whole. Additionally, this study provides a foundation for future pharmacological research to identify bioactive compounds of potential therapeutic value within this genus.

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REFERENCES

1. *Gymnosporia* (Wight & Arn.) Hook.f. | Plants of the World Online | Kew Science [Internet]. Plants of the World Online [cited 2024 Nov 10]; Available from: http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:1034880-2

2. Pham HH (2000), An illustrated flora of Vietnam [in Vietnamese], 2nd ed, Tre Publishing House, Ho Chi Minh City, Viet Nam, 151-153.

3. Nguyen TTH, Truong BN, Pham VC, et al (2014), Chemical constituents of the stems of *Gymnosporia stylosa* (Celastraceae) [in Vietnamese]. Vietnam Journal of Chemistry; 52:514–514. https://doi.org/10.15625/0866-7144.2014-0023

4. Tatsimo JSN, Toume K, Nagata T, Havyarimana L, Fujii T, Komatsu K (2019), Monoglycerol ester, galloylglucoside and phenolic derivatives from *Gymnosporia senegalensis* leaves. Biochemical Systematics and Ecology, 83:33–8.

5. Shahidi F, Ambigaipalan P (2015), Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. Journal of Functional Foods, 18:820–97.

6. Khoo HE, Prasad KN, Kong KW, Jiang Y, Ismail A. (2011), Carotenoids and their isomers: color pigments in fruits and vegetables. Molecules, 16(2):1710–38.

7. Pedroso APD, Santos SC, Steil AA, Deschamps F, Barison A, Campos F, et al. (2008), Isolation of syringaldehyde from *Mikania laevigata* medicinal extract and its influence on the fatty acid profile of mice. Rev bras farmacogn, 18:63–69.

8. Rout L, Nath P, Punniyamurthy T (2007), Vanadium-Catalyzed Selective Oxidation of Alcohols to Aldehydes and Ketones with tert-Butyl Hydroperoxide. Advanced Synthesis & Catalysis, 349:846–848.

9. Bui TTL, Dang HP, Nguyen TN (2015), Investigation of chemical constituents from the chloroform extract of the stem of *Paramignya trimera* (Oliver) Burkill (Rutaceae). in Pro-ceeding of the Conference of Analytical Chemistry, Valencia, Spain.

10. Kim, Jung Mi, 고려경, 현진원, Lee, Nam Ho (2009), Identification of New Dibenzofurans from Distylium racemosum. Bulletin of the Korean Chemical Society, 30(1):261–263.

11. Ouete JLN, Sandjo LP, Kapche DWFG, Liermann JC, Opatz T, Simo IK, et al (2013), A new flavone from the roots of *Milicia excelsa* (Moraceae). Z Naturforsch C J Biosci, 68(7–8):259–63.

12. Kieu TPL, Nguyen HQ, Nguyen VC, Nguyen QT, Vu HT, Nguyen VT, Nguyen PT (2020), Secondary metabolites from the stem barks of *Rhizophora mucronata* Lamk. Vietnam Journal of Science and Technology, 58(6): 653-664.

13. Q.X.Wu, Y.Li, Y-P.Shi (2024), Antioxidant phenolic glucosides from *Gentiana piasezkii.* Journal of Asian Natural Products Research, 8(5): 391-396.

14. Wasana Prapalert, Dammrong Santiarworn, Saisunee Liawruangrath, Boonsom Liawruangrath and Stephen G.Pyne (2016), The Isolation of Lutein and Lutein 3′-methyl ether from *Peristrophe lanceolaria*. Natural Product Communications, 11(12): 1793-1795.

15. Wu J, Fu YS, Lin K, Huang X, Chen Y jing, Lai D, et al (2022), A narrative review: The pharmaceutical evolution of phenolic syringaldehyde. Biomedicine & Pharmacotherapy, 153:113339.

16. Niquini FM, Tenorio JC, da Silva MFGF, Ribeiro AB, Wanderley A, Ellena J, et al (2020), On the conformation, molecular interactions and electron density of a natural flavonoid derivative. Journal of Molecular Structure, 1220:128632.

17. Posri P, Suthiwong J, Takomthong P, Wongsa C, Chuenban C, Boonyarat C, et al (2019), A new flavonoid from the leaves of *Atalantia monophylla* (L.) DC. Natural Product Research, 33(8):1115–1121.

18. Mitra S, Rauf A, Tareq AM, Jahan S, Emran TB, Shahriar TG, et al (2021), Potential health benefits of carotenoid lutein: An updated review. Food and Chemical Toxicology, 154:112328.

19. J-H Ha et al (2000), 4 Hydroxybenzaldehyde from *Gastrodia elata* B1 is active in the antioxidation and GABAergic neuromodulation of the rat brain. Journal of Ethnopharmacology, 73(1_2): 329-333.

20. Paul V, Tripathi A, Rai D, T Selvaraj R, Srivastava S (2021), A comprehensive review on vanillin: its microbial synthesis, isolation and recovery. Food Biotechnology, 35:22–49.

21. Nguyen TM, Le XT, Nguyen MTK (2017), Chemical constituents of *Breynia vitis-idaea* (Burm. f.) C. E. C. Fischer. AIP Conference Proceedings, 1878(1):020041.