Isolation and structural determination of pentacyclic triterpenoids from the leaves of *Gymnosporia chevalieri* tard

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Abstract

Background: The genus *Gymnosporia*, belonging to the Celastraceae family, which comprises approximately 116 species globally, with 8 species identified in Vietnam. This work initially describes the extraction, isolation, and structural identification of six triterpenoids from *G. chevalieri* collected in Vietnam. **Materials and methods:** The leaves of *G. chevalieri* were subjected to extraction through immersion, followed by a liquid-liquid partition process using organic solvents. Compounds were isolated using a combination of thin-layer chromatography and column chromatography. Their structures were determined based on 1D-, 2D-NMR as well as by comparison with the reported spectroscopic data. **Results & Conclusion:** The chemical constituents of *G. chevalieri* was reported for the first time. Six pentacyclic triterpenoids have been isolated and determined including mixture of *α*-amyrin (**1a**) and *β*-amyrin (**1b**), *β*-amyrenonol (**2**), 3-oxofriedelan-29-ol (**3**), taraxastane-3*β*,20*R*-diol (**4**), and taraxastane-3*β*,20*S*-diol (**5**).

Keywords: *Gymnosporia chevalieri, α-amyrin, β-amyrin, β-amyrenonol, 3-oxofriedelan-29-ol, taraxastane-3,20-diol.*

1. INTRODUCTION

The genus *Gymnosporia* (Celastraceae family) comprises approximately 116 species worldwide [1]. Among these, eight species have been identified in Vietnam, including *G. diversifolia, G. stylosa, G. bonii, G. chevalieri, G. gracilis, G. marcanii, G. mekongensis*, and *G. tonkinensis* [2]. Although phytochemical studies on the genus *Gymnosporia* globally began in the 1970s and have yielded impressive results, domestic scientific interest in this resource has only emerged in recent years, with a few publications currently available on *G. stylosa* (commonly known as "Dây lóp bóp") [3], [4], [5].

Gymnosporia chevalieri ("Lõa châu" Chevalier) is an endemic species in Vietnam. The chemical constituents and biological activities of this species remains relatively novel to scientific communities. This article presents, for the first time, the extraction, isolation, and structural determination of six pentacyclic triterpenoids from the *n*-hexane extract of the leaves of *Gymnosporia chevalieri*.

2. MATERIALS AND METHODS

2.1. Materials

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

2.2. Methods

The powdered material was extracted with methanol (MeOH) using maceration at room temperature. The obtained crude extracts were fractionated using liquid-liquid partitioning with *n*-hexane, ethyl acetate (EtOAc). Pure compounds were isolated by thin-layer chromatography (TLC) and column chromatography (CC). TLC was performed on pre-coated DC-Alufolien 60 F_{254} and RP_{18} F_{254} plates (Merck, Germany). Compounds were detected under UV light at wavelengths of 254 and 365 nm or by spraying the plates with 10% H_2SO_4 reagent followed by heating until color development. Column chromatography was carried out using various 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 structures of the compounds were determined based on ^{1}H-, ^{13}C-NMR, HSQC, and HMBC

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spectra, combined with comparison to reference spectral data. NMR spectra were recorded using a Bruker Avance Neo 600 Spectrometer (Bruker, Massachusetts, USA) at the Institute of Chemistry, Vietnam Academy of Science and Technology. $CDCl_3$ was used as the solvent, and tetramethylsilane (TMS) served as the internal standard.

3. RESULTS AND DISCUSSION

3.1. Extraction and Isolation

The leaves of *Gymnosporia chevalieri* were cleaned, air-dried, and ground into powder (4 kg). 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 (GCM, 930 g). This extract was partitioned into water (2 L) and successively extracted with *n*-hexane and EtOAc (each, 4 L x 3 times). The solvents were evaporated under reduced pressure to obtain the corresponding extracts: *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 GCH3 (109.1 g) was repeatedly washed with acetone to yield compound **1** (97.0 g). 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 GCH5.1 (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 GCH5.1.5 (31.3 mg) was purified by RP-18 column using acetone/water (20:1, v/v) to yield **3** (20 mg).

Fraction GCH5.1.4 (301.4 mg) was sequentially separated by sephadex column using CH_2Cl_2/MeOH (1:1, v/v), followed by RP-18 column using acetone/water (10:1, v/v), yielding five fractions: GCH5.1.4.1-GCH5.1.4.5. Fraction GCH5.1.4.5 (54.1 mg) was purified by sephadex column using CH_2Cl_2/MeOH (1:1, v/v), yielding compound **2** (6.7 mg).

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 GCH5.1.6.1 (300 mg) was further separated by sephadex column using CH_2Cl_2/MeOH (1:1, v/v), yielding three fractions: GCH5.1.6.1.1-GCH5.1.6.1.3. Fraction GCH5.1.6.1.2 (209 mg) was further separated by silica gel column using CH_2Cl_2/acetone (40:1, v/v), yielding five fractions: GCH5.1.6.1.2.1-GCH5.1.6.1.2.5. Fraction GCH5.1.6.1.2.3 (45.5 mg) was purified by RP-18 column using acetone/water (10:1, v/v), yielding compound **4** (6.7 mg). Fraction GCH5.1.6.1.2.5 (19 mg) was purified by RP-18 column using acetone/water (5:1, v/v), yielding compound **5** (3.5 mg).

3.2. Structural determination of isolates

Compound **1** was isolated as a white powder. The ^{1}H-NMR spectrum indicated characteristic signals of two olefinic protons at δ_H 5.18/5.13 (t, J = 3.6 Hz); two oxymethine groups at δ_H 3.23/3.22 (dd, J = 10.8, 4.8 Hz); and 16 methyl groups at δ_H 1.14, 1.07, 1.01, 1.00 (6H), 0.97, 0.95, 0.94, 0.92, 0.87 (6H), 0.83, 0.80, and 0.79 (9H). These data suggest that compound **1** is a mixture of two triterpenoids (**1a**:**1b**) (Figure 1).

The ^{13}C-NMR and HSQC spectra revealed the presence of two oxymethine groups (δ_C 79.1/79.0) and two trisubstituted double bonds (δ_C 139.6 (C)/124.5 (CH) and 145.2 (C)/121.8 (CH)), which were attributed to **1a** and **1b**, respectively. The assignment of NMR data for components **1a** and **1b** was based on detailed analysis of the HMBC spectrum of compound **1** (Table 1). Particularly, the position of the OH group at C-3 of each compound was confirmed by the correlations of H_3-23 (δ_H 1.00)/H_3-24 (δ_H 0.79) with C-3 (δ_C 79.1/79.0), C-4 (δ_C 38.8/39.7), and C-5 (δ_C 55.2). The position of the double bond at Δ^{12} was established by HMBC correlations between H-12 (δ_H 5.13) and C-9 (δ_C 47.8)/C-14 (δ_C 41.6)/C-18 (δ_C 59.1) for **1a**, and between H-12 (δ_H 5.18) and C-9 (δ_C 47.7)/C-14 (δ_C 42.1)/C-18 (δ_C 47.3) for **1b**.

The correlation of two methyl doublets [δ_H 1.01 (H_3-29), 0.92 (H_3-30)] with C-19 (δ_C 39.6)/C-20 (δ_C 38.8) confirmed the ursane skeleton for **1a**. Meanwhile, the correlation of two methyl singlets [δ_H 0.87 (6H, H_3-29 & H_3-30)] with C-19 (δ_C 46.9)/C-20 (δ_C 31.1)/C-21 (δ_C 34.8) suggested an oleanane skeleton for **1b** (Figure 2). Based on the above data, compound **1** was identified as a mixture of α-amyrin (**1a**) and β-amyrin (**1b**) [6]. The ratio of **1a**:**1b**, approximately 1.25:1, was determined from the integration values of the characteristic H-12 signals at δ_H 5.18 (for **1a**) and 5.13 (for **1b**).

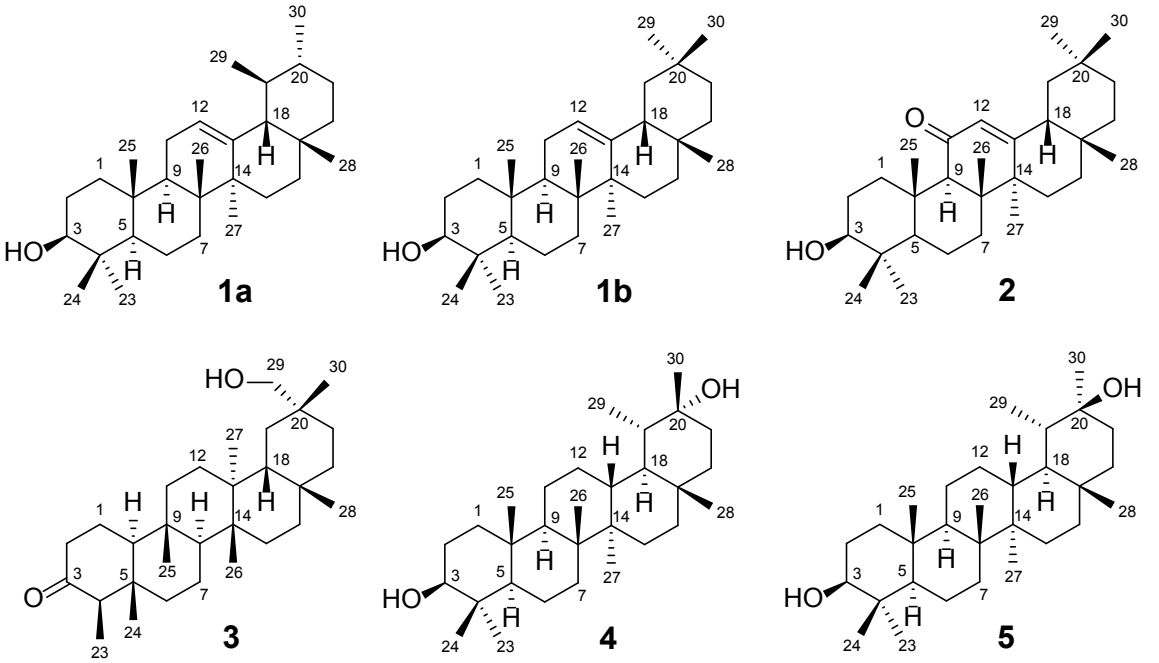

Figure 1. Structures of pentacyclic triterpenoids isolated from *Gymnosporia chevalieri*

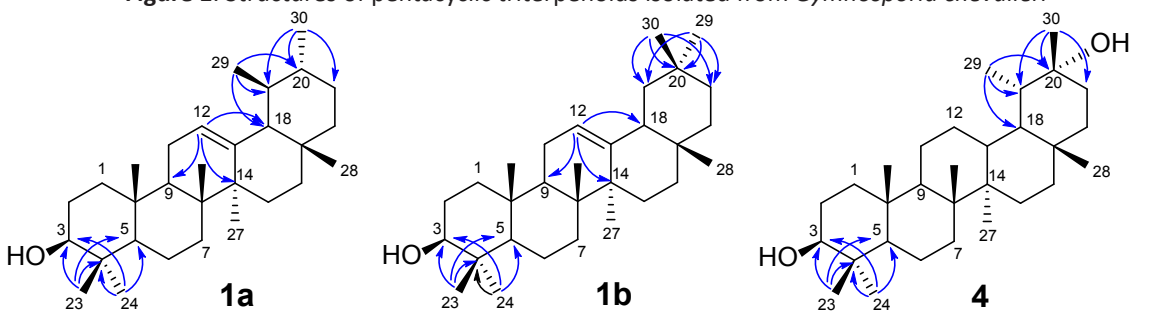

Figure 2. Selected HMBC correlations of **1a**, **1b** and **4**

Compound **2** was isolated as a white powder, readily soluble in CH_2Cl_2. The ^{1}H-NMR spectrum displayed a characteristic olefinic proton signal at δ_H 5.58 (s) and a carbinol group signal at δ_H 3.22 (dd, J = 11.4, 6.0 Hz). In addition, the high-field region revealed eight singlet methyl group signals at δ_H 1.36, 1.14, 1.13, 1.00, 0.90, 0.89, 0.86, and 0.81. The ^{13}C-NMR and HSQC spectra of the compound showed 30 carbon signals, including 8 CH_3, 9 CH_2, 5 CH, and 8 quaternary carbons (Table 2).

Notably, 1D-NMR data confirmed the presence of several key functional groups, including a carbonyl group (δ_C 200.3), a trisubstituted double bond (δ_C/δ_H 170.6, 128.1/5.58), and a carbinol group (δ_C/δ_H 78.8/3.22). The strong downfield shift of the sp^2 carbon (δ_C 170.6) and the singlet proton signal of the olefin (δ_H 5.58) suggested the conjugation of the double bond with a ketone group, forming an enone structure. These spectral data indicated that compound **2** is a pentacyclic triterpenoid with an oleanane skeleton similar to *β*-amyrin (**1b**). Further comparison with reference spectra [7] allowed the identification of compound **2** as 3*β*-hydroxy-11-oxo-olean-12-ene, also known as *β*-amyrenonol or 11-oxo-*β*-amyrin.

The ^{1}H-NMR signals of compound **3** mainly appeared in the high-field region (δ_H 0.7-3.5), typical for saturated terpenoids. Notable resonance signals included an oxymethylene group [δ_H 3.26 (2H, q, J = 10.2 Hz)], six methyl singlets [δ_H 1.22, 1.05, 1.04, 1.03, 0.87, and 0.73], and one methyl doublet [δ_H 0.88 (d, J = 6.6 Hz)].

The ^{13}C-NMR spectrum revealed 30 carbon signals, with notable features including a carbonyl group (δ_C 200.3) and an oxymethylene group (δ_C 74.8). HSQC data allowed classification of the 30

carbons into 7 CH_3 (δ_C 32.1, 25.8, 20.8, 18.5, 17.9, 14.7, 6.8), 12 CH_2 (δ_C 74.8, 41.5, 41.3, 39.5, 35.9, 35.6, 32.7, 30.6, 30.5, 27.8, 22.3, 18.2), 4 CH (δ_C 59.5, 58.2, 53.4, 41.9), and 7 C (δ_C 213.2, 42.2, 40.0, 38.2, 37.4, 33.1, 29.8). The methyl doublet signal at δ_C 6.8, which is diagnostic of triterpenoids with a friedelane skeleton, further supports this structural assignment. After comparison with reference spectral data [8], compound **3** was identified as 3-oxofriedelan-29-ol.

Table 1. NMR data of mixture **1a**:**1b** and reference compounds

C	1a			1b		
	$\delta_C^{\#, a}$	$\delta_C^{a, b}$	$\delta_H^{a, c}$ (*J*, Hz)	$\delta_C^{*, a}$	$\delta_C^{a, b}$	$\delta_H^{a, c}$ (*J*, Hz)
1	38.8	38.8 t	1.63 m/0.98 m	38.6	38.6 t	1.63 m/0.98 m
2	27.3	27.3 t	1.60 m/1.57 m	27.2	27.3 t	1.60 m/1.57 m
3	79.1	79.1 d	3.23 dd (10.8; 4.8)	79.0	79.0 d	3.22 dd (10.8; 4.8)
4	38.8	38.8 s	-	39.8	39.7 s	-
5	55.2	55.2 d	0.74 m	55.2	55.2 d	0.74 m
6	18.4	18.4 t	1.55 m/1.40 m	18.4	18.4 t	1.55 m/1.40 m
7	32.9	33.0 t	1.54 m/1.35 m	32.5	32.5 t	1.54 m/1.35 m
8	40.0	40.0 s	-	41.7	41.8 s	-
9	47.7	47.8 d	1.52 m	47.6	47.7 d	1.55 m
10	36.9	36.9 s	-	37.0	37.0 s	-
11	23.3	23.3 t	1.63 m/0.98 m	23.7	23.7 t	1.88 m/1.85 m
12	124.4	124.5 d	5.13 t (3.6)	121.7	121.8 d	5.18 t (3.6)
13	139.6	139.6 s	-	145.2	145.2 s	-
14	41.5	41.6 s	-	42.8	42.1 s	-
15	28.1	28.1 t	2.00 m/0.86 m	26.9	27.0 t	2.00 m/0.80 m
16	26.6	26.6 t	1.83 m/0.96 m	26.2	26.2 t	1.77 td (13.2; 4.2)/0.96 m
17	33.8	33.8 s	-	32.7	32.7 s	-
18	59.1	59.1 d	1.32 m	47.2	47.3 d	1.95 m
19	39.7	39.6 d	1.32 m	46.8	46.9 t	1.67 m/1.00 m
20	39.6	38.8 d	0.87 m	31.1	31.1 s	-
21	31.3	31.3 t	1.38 m/1.26 m	34.7	34.8 t	1.33 m/1.10 m
22	40.0	39.8 t	1.42 m/1.27 m	37.1	37.2 t	1.43 m/1.22 tt (10.2; 3.0)
23	28.1	28.1 q	1.00 s	28.1	28.1 q	1.00 s
24	15.7	15.7 q	0.79 s	15.6	15.6 q	0.79 s
25	15.6	15.6 q	0.95 s	15.5	15.5 q	0.94 s
26	17.4	17.5 q	0.79 s	16.8	16.8 q	0.97 s
27	23.4	23.4 q	1.07 s	26.0	26.0 q	1.14 s
28	28.8	28.8 q	0.80 s	28.4	28.4 q	0.83 s
29	16.9	16.9 q	1.01 d (6.0)	33.3	33.3 q	0.87
30	21.4	21.4 q	0.92 d (6.0)	23.5	23.6 q	0.87 s

$^{\#, *}\delta_C$ values of α-, β-amyrin [6], ^{a}measured in $CDCl_3$, ^{b}150 MHz, ^{c}600 MHz

Table 2. NMR data of **2, 3** and reference compounds

C	2			3		
	$\delta_C^{\#,a}$	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (*J*, Hz)	$\delta_C^{*,a}$	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (*J*, Hz)
1	38.7	39.2 t	2.78 dt (13.2; 3.6)/0.98 m	22.3	22.3 t	1.95 m/1.67 m
2	23.4	27.3 t	1.66 m/1.62 m	41.6	41.5 t	2.38 m/2.29 m
3	80.5	78.8 d	3.22 dd (11.4; 6.0)	212.2	213.2 s	-
4	38.0	39.2 s	-	58.3	58.2 d	2.24 ddd (7.2; 5.4; 1.8)
5	54.9	55.0 d	0.69 dd (12.0; 1.2)	42.2	42.2 s	-
6	18.4	17.5 t	1.59 m/1.45 m	41.4	41.3 t	1.75 m/1.28 m
7	32.6	32.8 t	1.65 m/1.42 m	18.3	18.2 t	1.49 m/1.40 m
8	43.3	43.4 s	-	53.5	53.4 d	1.40 m
9	61.5	61.8 d	2.34 s	37.5	37.4 s	-
10	36.9	37.1 s	-	59.6	59.5 d	1.52 m
11	201.4	200.3 s	-	35.7	35.6 t	1.45 m/1.26 m
12	127.9	128.1 d	5.58 s	29.8	30.5 t	1.35 m
13	170.2	170.6 s	-	40.0	40.0 s	-
14	45.3	45.4 s	-	38.3	38.2 s	-
15	26.4	26.5 t	2.06 td (13.2; 4.2) 1.82 td (13.2; 4.8)	32.8	32.7 t	1.55 m/1.32 m
16	26.4	26.4 t	1.17 m/0.96 m	36.0	35.9 t	1.59 m/1.33 m
17	32.3	32.4 s	-	29.8	29.8 s	-
18	47.5	47.6 d	2.13 dd (13.8; 3.6)	42.0	41.9 d	1.61 m
19	45.0	45.2 t	1.67 m/1.06 m	30.6	30.6 t	1.35 m
20	31.0	31.1 s	-	33.2	33.1 s	-
21	34.4	34.5 t	1.35 m/1.17 m	27.9	27.8 t	1.37 m
22	36.4	36.5 t	1.47 m/1.29 m	39.6	39.5 t	1.41 m/1.36 m
23	28.0	28.1 q	1.00 s	6.8	6.8 q	0.88 d (6.6)
24	16.6	16.4 q	1.14 s	14.7	14.7 q	0.73 s
25	15.7	15.6 q	0.81 s	17.9	17.9 q	0.87 s
26	17.3	18.7 q	1.13 s	18.4	18.5 q	1.05 s
27	23.5	23.5 q	0.89 s	20.8	20.8 q	1.04 s
28	28.7	28.8 q	0.86 s	32.1	32.1 q	1.22 s
29	33.0	33.1 q	0.90 s	74.8	74.8 t	3.26 q (10.2)
30	23.5	23.5 q	1.36 s	25.9	25.8 q	1.03 s

$^{\#}\delta_C$ values of β-amyrenyl *acetate [7], $^{*}\delta_C$ values of 3-oxofriedelan-29-ol [8], [a]measured in $CDCl_3$, [b]150 MHz, [c]600 MHz*

Table 3. NMR data of **4**, **5** and reference compounds

C	4			5			$\Delta\delta_C$
	δ_C [#, a]	δ_C [a, b]	δ_H [a, c] (*J*, Hz)	δ_C [*, a]	δ_C [a, b]	δ_H [a, c] (*J*, Hz)	
1	38.7	38.7 t	1.69 m/0.93 m	38.6	38.6 t	1.68 m/0.92 m	0.1
2	27.4	27.4 t	1.61 m/1.57 m	27.4	27.4 t	1.62 m/1.57 m	0
3	79.0	79.1 d	3.19 dd (12.0; 4.8)	79.0	79.1 d	3.20 dd (12.0; 4.8)	0
4	38.8	38.8 s	-	38.8	38.8 s	-	0
5	55.1	55.1 d	0.68 m	55.1	55.1 d	0.68 m	0
6	18.3	18.3 t	1.52 m/1.37 m	18.4	18.4 t	1.52 m/1.36 m	0
7	34.5	34.5 t	1.39 m/1.36 m	34.4	34.4 t	1.39 m/1.36 m	0.1
8	41.4	41.4 s	-	41.2	41.3 s	-	0.1
9	49.6	49.6 d	1.22 m	49.4	49.5 d	1.25 m	0.1
10	36.9	37.0 s	-	36.9	36.9 s	-	0.1
11	21.6	21.6 t	1.47 m/1.24 m	21.3	21.3 t	1.45 m/1.25 m	0.3
12	29.4	29.4 t	1.77 m/1.25 m	28.5	28.5 t	1.98 m/1.17 m	0.9
13	39.0	39.0 d	1.78 m	38.8	38.8 d	1.83 m	0.2
14	43.2	43.2 s	-	43.0	43.0 s	-	0.2
15	26.6	26.6 t	1.77 m/0.98 m	26.5	26.5 t	1.74 m/1.97 m	0.1
16	38.2	38.2 t	1.36 m/1.15 m	38.3	38.3 t	1.32 m/1.18 m	0.1
17	35.1	35.2 s	-	35.6	35.6 s	-	0.4
18	47.4	47.5 d	1.19 m	47.9	47.9 d	1.04 m	0.4
19	38.8	38.8 d	1.34 m	42.0	42.0 d	1.52 m	**3.2**
20	73.6	73.6 s	-	75.3	75.3 s	-	**1.7**
21	35.5	35.5 t	1.72 m/1.47 m	37.8	37.8 t	1.70 m/1.51 m	**2.3**
22	37.8	37.8 t	1.50 m/1.08 m	40.2	40.2 t	1.23 m	**2.4**
23	28.0	28.0 q	0.97 s	28.1	28.0 q	0.97 s	0
24	15.4	15.4 q	0.76 s	15.5	15.4 q	0.76 s	0
25	16.2	16.2 q	1.05 s	16.1	16.1 q	0.84 s	0.1
26	16.2	16.2 q	0.84 s	16.0	16.0 q	1.04 s	0.2
27	14.8	14.8 q	0.95 s	14.7	14.7 q	0.94 s	0.1
28	17.8	17.8 q	0.83 s	18.4	18.4 q	0.90 s	0.6
29	17.9	17.9 q	1.06 d (6.0)	17.4	17.4 q	1.06 d (6.0)	0.5
30	30.3	30.3 q	1.18 s	21.4	21.4 q	1.08 s	**8.9**

*[#]δ_C values of taraxastane-3β,20R-diol, [*]δ_C values of taraxastane-3β,20S-diol [9], [a]measured in $CDCl_3$, [b]150 MHz, [c]600 MHz; $\Delta\delta_C = |\delta_C$ of **4** - δ_C of **5**|.*

Compound **4** was isolated as a white powder, soluble in CH_2Cl_2. The ^{1}H-NMR spectrum of compound **4** exhibited a characteristic resonance for an oxymethine proton at δ_H 3.19 (dd, *J* = 12.0; 4.8 Hz), along with seven singlet methyls at δ_H 1.18, 1.05, 0.97, 0.95, 0.84, 0.83, and 0.76, and a doublet methyl at δ_H 1.06 (d, *J* = 6.0 Hz).

The ^{13}C-NMR spectrum showed resonances for 30 sp^3 carbons (in the range δ_C 14-79 ppm). The HSQC technique classified these signals into eight methyl, ten methylene, six methine groups, and six quaternary carbons. Two oxygen-bearing

carbons were observed at δ_C 79.1 (CH) and 73.6 (C), suggesting that compound **4** is a triterpene diol.

The HMBC correlations of H_3-23 (δ_H 0.97)/H_3-24 (δ_H 0.76) with C-3 (δ_C 79.1)/C-4 (δ_C 38.8)/C-5 (δ_C 55.1) confirmed that the hydroxyl group is attached to C-3. Furthermore, the correlations of the doublet methyl H_3-29 (δ_H 1.06) with C-18 (δ_C 47.5)/C-19 (δ_C 38.8)/C-20 (δ_C 73.6), and of H_3-30 (δ_H 1.18) with C-19/C-20/C-21 (δ_C 35.5), demonstrated that the other hydroxyl group is attached to C-20. The presence of the 20-OH group causes a strong downfield shift of the C-30 methyl group (δ_C/δ_H 30.3/1.18) compared to the other methyl groups in **4**. By comparing these data with the NMR spectrum of a reference compound [9], compound **4** was identified as taraxastane-3*β*,20*R*-diol.

Compound **5** showed very similar NMR data to compound **4**, except for the signals corresponding to C-20 and adjacent carbons. Specifically, the δ_C values for positions C-19, C-20, C-21, C-22, and C-30 between the two compounds differed by 3.2, 1.7, 2.3, 2.4, and 8.9 ppm, respectively, while the remaining positions exhibited deviations of less than 1 ppm (Table 3). Based on these differences, compound **5** was proposed to be taraxastane-3*β*,20*S*-diol, the 20-epimer of compound **4**. This conclusion was further confirmed by comparison with the NMR data of a reference compound [9].

All six compounds were isolated for the first time from *Gymnosporia chevalieri*. The presence of *α*- and *β*-amyrin as a mixture that cannot be separated by conventional chromatographic techniques has been reported in numerous previous studies. For instance, this mixture has been found in *Celastrus hindsii* [6], *Byrsonima crassifolia* [10], *Byrsonima fagifolia* [11], and *Pouteria gardnerii* [12]. In this study, the *α*-, *β*-amyrin mixture was identified as a major chemical component of *G. chevalieri* leaves, with a preliminary estimated content of about 25 g/kg of dry material. Therefore, *G. chevalieri* may be considered a potential source of *α*-, *β*-amyrin for future applications [13]. *β*-Amyrin has also been previously isolated from *Gymnosporia stylosa* [3]. *β*-Amyrenonol (**2**) has been found in *Maytenus cuzcoina* (Celastraceae), a species closely related to the *Gymnosporia* genus [14]. While taraxastane-3,20-diol (**4**, **5**) has been isolated for a long time, the absolute configuration at C-20 was ambiguous and has only recently been fully resolved through NOESY analysis combined with theoretical calculations [9].

Regarding biological activities of isolates, *α*- and *β*-amyrin have been shown to exhibit numerous potential *in vitro* and *in vivo* pharmacological effects, including anti-inflammatory, antibacterial, antifungal, antiviral, and anticancer activities [13]. *β*-Amyrin (**1b**) demonstrated the strongest antifungal activity with an MIC value of 0.0156 mg/mL [13]. *β*-Amyrenonol (**2**), an oleanolic triterpenoid isolated from licorice roots, is a precursor of glycyrrhetinic acid and exhibits antiproliferative and anti-inflammatory activities [15], [16]. It also inhibited the growth of HL60 cells with an IC_{50} value of 26.3 μM and significantly reduced lipopolysaccharide-induced TNFα release in THP-1 cells [16]. Taraxastane-3*β*,20*R*-diol (**4**) showed strong inhibition of Epstein–Barr virus early antigen induction stimulated by 12-*O*-tetradecanoylphorbol-13-acetate in Raji cells [17].

4. CONCLUSION

The chemical composition of *Gymnosporia chevalieri* is reported for the first time. Six pentacyclic triterpenoids, including a mixture of *α*-amyrin (**1a**) and *β*-amyrin (**1b**), *β*-amyrenonol (**2**), 3-oxofriedelan-29-ol (**3**), taraxastane-3*β*,20*R*-diol (**4**), and taraxastane-3*β*,20*S*-diol (**5**), have been isolated from the *n*-hexane extract of *G. chevalieri* leaves. Their structures were determined based on 1D and 2D NMR analyses and by comparison with previously reported spectroscopic data. These isolates have shown valuable biological effects in the literature. Therefore, further biological activity tests should be conducted to identify potential active agents for pharmaceutical applications.

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