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TRITERPENOID CONSTITUENTS FROM THE LEAVES OF *WALSURA PINNATA* HASSK

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Summary

By employing chromatographic techniques, six triterpenoids (**1**−**6**) were successfully isolated from the leaves of *Walsura pinnata* Hassk. Their structures were elucidated to be cumingianol E (**1**), 21-*O*-methyltoosendanpentol (**2**), 21*α*-methylmelianodiol (**3**), 21*S*,23*R*-epoxy-3*β*,24*R*-dihydroxy-21*β*,25-dimethoxy-tirucalla-7-en (**4**), odoratone (**5**), and cabraleone (**6**) by detailed analysis of 1D, 2D NMR, and MS spectroscopic data, as well as comparison with previously reported literature. This is the first time compounds **1**−**6** from *W. pinnata* were reported.

Keywords: *Walsura pinnata; Meliaceae; Apotirucallane; Tirucallane; Dammarane; Triterpenoids.*

1. Introduction

Plants of the genus *Walsura* Roxb, belonging to the Meliaceae family and comprising approximately 21 species [1], are distributed in subtropical regions, including South India, mainland China, and Southeast Asia (Malaysia, Indonesia, Thailand, and Vietnam) [2]. Currently, there are no accurate data regarding the exact number of *Walsura* species present in Vietnam. However, based on published literature, seven species have been recorded in the country: *W. bonii*, *W. cochinchinensis*, *W. elata*, *W. pinnata*, *W. poilanei*, *W. robusta*, and *W. villosa* [3]. Traditionally, members of this genus have been used in folk medicine to treat various ailments, such as tendon disabilities, hemorrhoids, excessive bleeding, and wounds, as well as for their expectorant, emmenagogue, and emetic properties [4]. Previous phytochemical investigations on *Walsura* have led to the isolation of phenols, limonoids, lignans, and terpenoids, some of which exhibited cytotoxic, antimicrobial, antioxidant, antifeedant, and neuroprotective activities [5],[6],[7],[8]. However, phytochemical and pharmacological data on *W. pinnata* remain limited [4]. To date, a few studies have reported the presence of sesquiterpenoid and triterpenoid constituents, which have drawn interest due to their cytotoxic effects [9],[10],[11]. In continuation of our interest in the phytochemistry of plants belonging to the genus *Walsura*, we have conducted further investigations and successfully isolated six triterpenoid compounds (**1**−**6**, Fig. 1) from the leaves of *W. pinnata*.

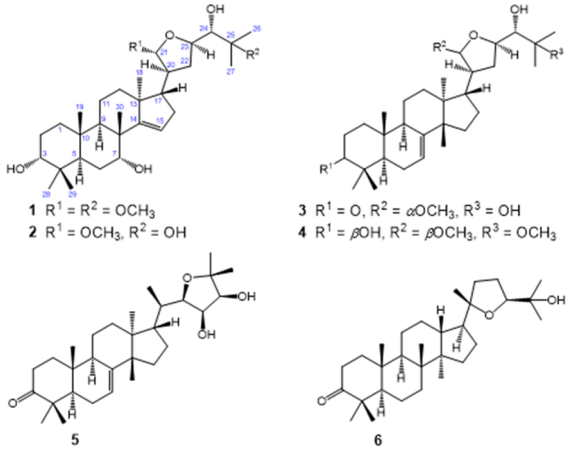

Fig. 1. Chemical structures of compounds **1**−**6**

2. Materials and methods

2.1. Chemicals and equipment

Optical rotations were determined on a JASCO P-2000 polarimeter. The NMR spectra were recorded on a Bruker AVANCE NEO 600 FT-NMR spectrometer with TMS as an internal standard. The HRESIMS data were taken on an Agilent 6530 Accurate Mass Q-TOF LC/MS. Column chromatography (CC) was conducted on *silica gel*, Diaion HP-20, Sephadex LH-20, and RP-18. Preparative HPLC was performed using Agilent 1100, J′ sphere ODS H-80 column (4

μm, 250 × 20 mm), a flow rate of 6 mL/min, and a detector DAD at wavelengths of 205, 230, 254, and 280 nm. An isocratic mobile phase with a flow rate of 3.0 mL/min was used in pre-HPLC. Analytical thin-layer chromatography was performed on precoated *silica gel* 60 F_{254} and RP-18 $F_{254}S$ plates, and compounds were visualized by spraying with 10% H_2SO_4 in water and then heating for 1.5−2 minutes.

2.2. Plant material

The leaves of *W. pinnata* were collected at Dakrong, Quang Tri Province, Vietnam, in May 2025. The species was taxonomically identified by Dr. Le Tuan Anh (Vietnam National Museum of Nature). A voucher herbarium specimen (NCCT-P184) has been deposited at the Herbarium of the Institute of Chemistry, VAST.

2.3. Extraction and isolation

The dried leaves of *W. pinnata* (3.5 kg) were ultrasonically extracted with methanol (3 × 7.0 L, each for 2.5 h at room temperature), yielding a MeOH extract (300 g) after solvent removal under reduced pressure. The extract was then suspended in water and sequentially partitioned with organic solvents to obtain the *n*-hexane (H, 32.5 g), dichloromethane (D, 95 g), ethyl acetate (E, 40 g), and aqueous fractions. HPLC analysis indicated that the CH_2Cl_2 fraction contained a relatively high abundance of secondary metabolites, and this fraction was therefore selected for further chromatographic separation. The CH_2Cl_2 fraction (95 g) was subjected to *silica gel* CC, using a gradient solvent system of CH_2Cl_2-MeOH (from 50:0 to 0:100), based on TLC analysis results. Fractions with similar polarity were combined, resulting in the acquisition of seven fractions (D1−D7). Fraction D2 (5.1 g) was purified on an RP-18 column using 40% MeOH in H_2O as the eluent, yielding six subfractions (D2.1−D2.6). Among these, subfraction D2.2 (56 mg) was further purified by semi-preparative HPLC with CH_3CN-H_2O (65:35) as the mobile phase, yielding compound **1** (6.5 mg, t_R = 44.3 min). Similarly, subfraction D2.3 (82 mg) was purified by a semi-preparative HPLC system using a CH_3CN-H_2O (50:50) mixture, resulting in the isolation of compound **2** (10.8 mg, t_R = 33.8 min). Subfraction D3 (1.52 g) was subjected to RP-18 CC with MeOH-H_2O (2:3) as the eluent, producing three subfractions (D3.1−D3.3). Further purification of subfraction D3.2 (75 mg) by semi-preparative HPLC, using CH_3CN-H_2O (30:70) as the mobile phase, yielded compound **6** (7.2 mg, t_R = 47.3 min). Additionally, subfraction D3.3 (0.8 g) was subjected to *silica gel* CC using a CH_2Cl_2-MeOH-H_2O solvent system (5:1:0.1, v/v/v), yielding five fractions (D3.3a to D3.3e). Compounds **3** (10.1 mg, t_R = 31.8 min) and **5** (7.9 mg, t_R = 35.4 min) were obtained from subfraction D3.3b (0.11 g) through semi-preparative HPLC system (CH_3CN-H_2O containing 0.05% TFA, 20:80). In a similar process to that described above, subfraction D3.3d (0.10 g) was purified by semi-preparative HPLC using 20% CH_3CN in H_2O as the mobile phase, yielding compound **4** (8.9 mg, t_R = 40.5 min).

Cumingianol E (1): White amorphous powder; $[\alpha]_D^{23}$: −89.5 (*c* 0.1, $CHCl_3$); HRESIMS *m/z* 557.3840 $[M + Na]^+$ (calcd. for $[C_{32}H_{54}NaO_6]^+$, 557.3835); ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectroscopic data, see Table 1.

21-O-Methyltoosendanpentol (2): White amorphous powder; $[\alpha]_D^{23}$: −41.4 (*c* 0.1, $CHCl_3$); HRESIMS *m/z* 543.3679 $[M + Na]^+$ (calcd. for $[C_{31}H_{52}NaO_6]^+$, 543.3676); ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectroscopic data, see Table 1.

21α-Methylmelianodiol (3): White amorphous powder; $[\alpha]_D^{23}$: −89.7 (*c* 0.1, $CHCl_3$); HRESIMS *m/z* 503.3739 $[M + H]^+$ (calcd. for $[C_{31}H_{51}O_5]^+$, 503.3731); ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectroscopic data, see Table 2.

21S,23R-epoxy-3β,24R-dihydroxy-21β,25-dimethoxy-tirucalla-7-en (4): White amorphous powder; $[\alpha]_D^{23}$: +35.0 (*c* 0.1, $CHCl_3$); HRESIMS *m/z* 541.3874 $[M + Na]^+$ (calcd. for $[C_{32}H_{54}NaO_5]^+$, 541.3864); ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectroscopic data, see Table 2.

Odoratone (5): Colorless needles; $[\alpha]_D^{23}$: −41.8 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz) δ (ppm): 1.45 (1H, m, H_a-1), 1.98 (1H, m, H_b-1), 2.24 (1H, dd, *J* = 14.4, 1.6 Hz, H_a-2), 2.75 (1H, ddd, *J* = 14.4, 14.4, 5.4 Hz, H_b-2), 1.72 (1H, t, *J* = 9.0 Hz, H-5), 2.10 (2H, m, H-6), 5.31 (1H, dd, *J* = 6.6, 3.6 Hz, H-7), 2.28 (1H, m, H-9), 1.56 (2H, m, H-11), 1.62 (1H, m, H_a-12), 1.86 (1H, m, H_b-12), 1.50 (2H, m, H-15), 1.37 (1H, m, H_a-16), 2.02 (1H, m, H_b-16), 1.87 (1H, m, H-17), 0.83 (3H, s, H-18), 1.00 (3H, s, H-19), 1.64 (1H, m, H-20), 0.85 (3H, d, *J* = 7.2 Hz, H-21), 3.82 (1H, dd, *J* = 6.6, 1.8 Hz, H-22), 3.97 (1H, dd, *J* = 6.0, 5.4 Hz, H-23), 3.66 (1H, dd, *J* = 6.0, 5.4 Hz, H-24), 1.22 (3H, s, H-26), 1.22 (3H, s, H-27), 1.05 (3H, s, H-28), 1.11 (3H, s, H-29), and 1.03 (3H, s, H-30); ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm):

38.6 (C-1), 35.1 (C-2), 217.1 (C-3), 47.9 (C-4), 52.4 (C-5), 24.4 (C-6), 117.8 (C-7), 146.0 (C-8), 48.6 (C-9), 35.0 (C-10), 18.3 (C-11), 33.6 (C-12), 43.6 (C-13), 51.2 (C-14), 34.1 (C-15), 27.9 (C-16), 49.3 (C-17), 21.9 (C-18), 12.8 (C-19), 37.5 (C-20), 12.4 (C-21), 83.7 (C-22), 72.9 (C-23), 77.4 (C-24), 80.9 (C-25), 27.7 (C-26), 21.3 (C-27), 24.6 (C-28), 21.6 (C-29), and 27.7 (C-30).

Cabraleone (6): White amorphous powder; $[\alpha]_D^{23}$: +67.3 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz) δ (ppm): 1.45 (1H, m, H_a-1), 1.92 (1H, m, H_b-1), 2.43 (1H, m, H_a-2), 2.50 (1H, m, H_b-2), 1.37 (1H, dt, J = 9.0, 3.0 Hz, H-5), 1.47 (1H, m, H_a-6), 1.55 (1H, m, H_b-6), 1.32 (1H, m, H_a-7), 1.56 (1H, m, H_b-7), 1.43 (1H, m, H-9), 1.25 (1H, m, H_a-11), 1.52 (1H, m, H_b-11), 1.80 (1H, m, H_a-12), 1.88 (1H, m, H_b-12), 1.60 (1H, m, H-13), 1.10 (1H, m, H_a-15), 1.47 (1H, m, H_b-15), 1.25 (1H, m, H_a-16), 1.89 (1H, m, H_b-16), 1.82 (1H, m, H-17), 0.99 (3H, s, H-18), 0.94 (3H, s, H-19), 1.14 (3H, s, H-21), 1.63 (1H, m, H_a-22), 1.72 (1H, m, H_b-22), 1.48 (1H, m, H_a-23), 1.78 (1H, m, H_b-23), 3.73 (1H, dd, J = 7.8, 7.2 Hz, H-24), 1.21 (3H, s, H-26), 1.12 (3H, s, H-27), 1.08 (3H, s, H-28), 1.04 (3H, s, H-29), and 0.88 (3H, s, H-30); ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 39.9 (C-1), 34.1 (C-2), 218.1 (C-3), 47.4 (C-4), 55.3 (C-5), 19.7 (C-6), 34.6 (C-7), 40.3 (C-8), 50.2 (C-9), 36.9 (C-10), 22.1 (C-11), 26.1 (C-12), 43.1 (C-13), 50.1 (C-14), 31.4 (C-15), 27.5 (C-16), 49.5 (C-17), 15.1 (C-18), 16.0 (C-19), 86.3 (C-20), 23.6 (C-21), 35.7 (C-22), 25.7 (C-23), 83.3 (C-24), 71.4 (C-25), 27.4 (C-26), 24.3 (C-27), 26.7 (C-28), 21.0 (C-29), and 16.4 (C-30).

3. Results and discussion

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{32}H_{54}O_6$, corresponding to six degrees of unsaturation, based on its sodium adduct molecular ion peak at *m/z* 557.3840 $[M + Na]^+$ (calcd. for $[C_{32}H_{54}NaO_6]^+$, 557.3835) in the HRESIMS spectrum. The ^1H NMR spectrum of **1** exhibited signals for seven tertiary methyl groups [δ_H 0.85, 0.90, 1.05, 1.06, 1.15, and 1.24 (3H, s, for each)], five oxymethine protons [δ_H 3.40 (1H, br s, H-3), 3.90 (1H, t, J = 3.0 Hz, H-7), 4.80 (1H, d, J = 3.0 Hz, H-21), 4.21 (1H, ddd, J = 10.8, 4.8, 2.4 Hz, H-23), and 3.37 (1H, d, J = 2.4 Hz, H-24)], two methoxy groups (δ_H 3.23 and 3.35), and a characteristic olefinic proton signal [δ_H 5.42 (1H, d, J = 1.8 Hz, H-15). All 32 carbons in the molecular structure were resolved as individual resonances in the ^{13}C NMR spectrum and classified using HSQC spectroscopy as two olefinic carbons [δ_C 162.4 (C-14) and 119.1 (C-15)], one acetal carbon [δ_C 109.2 (C-21)], four sp^3 quaternary carbons [δ_C 37.0 (C-4), 44.4 (C-8), 37.7 (C-10), and 47.0 (C-13)], and one oxygen-bearing sp^3 quaternary carbon [δ_C 77.0 (C-25)] (Table 1). All these data, coupled with a literature survey, indicated that **1** is an apotirucallane-type triterpenoid and shares the same A–D ring framework as cumingianol E [12]. Four rings and one double bond account for five of the six degrees of unsaturation, indicating that the remaining degree of unsaturation must arise from an additional ring. A spin system, CH(17)–CH(20)–CH(21)–CH(24)–CH(23)–CH_2(22)–CH(20), determined from the ^1H–^1H COSY spectrum (Fig. 2), suggested that C-21 is connected to C-20, C-22, and C-23. This connectivity requires the presence of an additional five-membered ring (ring E) attached at C-17. This structural feature was confirmed by HMBC correlations of H-21 (δ_H 4.80) with C-17, C-20, C-22, and C-23 (δ_C 57.7, 46.2, 35.5, and 75.1, respectively) as well as correlations of H_a-22 (δ_H 1.60) and H_b-22 (δ_H 1.97) with C-17, C-20, C-23, and C-24 (δ_C 76.3). HMBC correlations from the proton signals at δ_H 1.15 (H_3-26) and 1.24 (H_3-27) to the carbon signals at δ_C 76.3 (C-24) and 77.0 (C-25), together with the methoxy signal (δ_H 3.23) correlating with C-25, supported the presence of an isopropyl methoxy group at C-25. In addition, HMBC cross-peaks between H-21 and C-23, and between a methoxyl signal (δ_H 3.35) and C-21, indicated a tetrahydrofuran moiety bearing a methoxy group at C-21. Furthermore, HMBC correlations of H_3-28 (δ_H 0.95) with C-3, C-4, C-5, and C-29, and of H_3-30 (δ_H 1.05) with C-7, C-8, C-9, and C-14 were consistent with the presence of OH-3, OH-7, and $\Delta^{14,15}$ substituents, respectively. The hydroxy groups at C-3 and C-7 were assigned as α-oriented based on the broad singlet of H-3 and the small coupling constant values of H-7 (J = 3.0 Hz) [12].

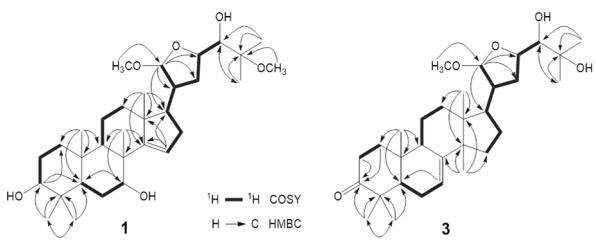

Fig. 2. Key COSY and HMBC correlations of **1** and **3**

Table 1. NMR spectroscopic data of compounds **1** and **2** and the reference compounds

No.	$^{\#}\delta_C$	**1**		$^{\$}\delta_C$ [a]	**2**	
		δ_C [a,b]	δ_H [a,c] mult. (*J* in Hz)		δ_C [a,b]	δ_H [a,c] mult. (*J* in Hz)
1	33.3	32.6	1.33 m/1.38 m	32.8	32.5	1.35 m/1.38 m
2	26.1	25.0	1.55 m/1.92 m	25.0	25.0	1.57 m/1.92 m
3	74.9	76.2	3.40 br s	76.2	76.1	3.39 br s
4	37.5	37.0	-	37.1	37.0	-
5	40.6	40.4	1.98 m	40.5	40.3	1.98 m
6	25.0	23.7	1.71 m	23.7	23.6	1.71 m
7	72.5	72.3	3.90 t (3.0)	72.3	72.3	3.91 br s
8	44.4	44.4	-	44.3	44.3	-
9	42.0	41.6	2.02 dd (12.0, 7.8)	41.6	41.6	2.02 dd (11.4, 7.2)
10	38.0	37.7	-	37.7	37.6	-
11	16.6	16.2	1.50 m/1.74 m	16.3	16.2	1.50 m/1.72 m
12	33.3	32.7	1.43 m/1.60 m	32.6	32.7	1.45 m/1.79 m
13	47.0	47.0	-	46.9	46.9	-
14	162.2	162.4	-	162.4	162.3	-
15	118.7	119.1	5.42 d (1.8)	119.2	119.1	5.43 br s
16	34.9	34.7	2.10 m/2.16 m	34.7	34.6	2.11 m/2.19 m
17	58.0	57.7	1.71 m	57.5	57.4	1.92 m
18	19.2	19.3	1.06 s	19.3	19.2	1.06 s
19	15.6	15.2	0.90 s	15.2	15.2	0.90 s
20	46.3	46.2	2.33 m	45.9	45.5	2.33 m
21	109.5	109.2	4.80 d (3.6)	109.6	109.4	4.80 d (3.0)
22	35.2	35.5	1.60 dd (12.0, 10.8) 1.97 ddd (12.0, 7.2, 4.8)	33.8	33.8	1.78 dd (12.0, 10.2) 1.95 ddd (12.0, 8.4, 4.8)
23	68.9	75.1	4.21 ddd (10.8, 4.8, 2.4)	76.9	76.7	4.24 ddd (10.2, 8.4, 4.8)
24	76.4	76.3	3.37 d (2.4)	75.6	75.6	3.24 d (4.8)
25	76.4	77.0	-	73.1	73.0	-
26	22.8	20.1	1.15 s	26.4	26.2	1.26 s
27	20.3	21.6	1.24 s	26.5	26.4	1.29 s
28	29.0	28.0	0.95 s	28.1	28.0	0.95 s
29	22.5	22.1	0.85 s	22.1	22.1	0.85 s
30	28.3	27.7	1.05 s	27.7	27.6	1.05 s
21-OCH_3	55.0	55.4	3.35 s	55.6	55.5	3.35 s
25-OCH_3	49.0	49.1	3.23 s			

Measured in [a]$CDCl_3$, [b]150 MHz, and [c]600 MHz. [#]δ_C of cumingianol E in pyridine-d_5 + D_2O [12] *and [$]$\delta_C$ of 21-O-methyltoosendanpentol in $CDCl_3$* [13].

Moreover, the *S*-configuration at C-24 was confirmed on the basis of the small coupling constant ($J_{H\text{-}23/H\text{-}24}$ = 2.4 Hz) in the ^{1}H NMR spectrum [12],[13]. The NOESY correlations of H_3-29/H-2β, H-2β/H_3-19, H_3-19/H_3-30, and H_3-30/H-17 indicated that these methyl groups are cofacial and β-oriented, whereas the correlations of H_3-28/H-5 and H-9/H_3-18 supported an α-orientation. Additionally, the cross-peak observed for H_3-18/H-20, H_a-22/H-23, and H-23/H_3-26 showed that both H-20 and H-23 are α-oriented, whereas those for H-17/H-21, H-21/H-24 revealed that H-21 and H-24 are β-oriented. Based on the above evidence and comparison with the published data [12], compound **1** was finally identified as cumingianol E (Fig. 1 and Table 1). This compound was previously obtained from the leaves of *Dysoxylum cumingianum* (Meliaceae) and was reported to show no growth inhibitory effects against KB, MCF-7, and KB-C2 cell lines [12].

Compound **2** was isolated as a white amorphous powder. The HRESIMS data of **2** showed a sodium adduct ion at *m/z* 543.3679 [M + Na]$^+$ (calcd. for $[C_{31}H_{52}NaO_6]^+$, 543.3676), consistent with the molecular formula $C_{31}H_{52}O_6$ (six degrees of unsaturation). Analysis of the NMR spectra allowed the assignment of most functional groups characteristic of an apotirucallane-type triterpenoid skeleton [12],[13]. Complete elucidation of 1D NMR (Table 1) and HSQC spectra indicated that the structure of **2** closely resembled that of cumingianol E (**1**) [12] (Table 1), except for minor differences observed at C-25 resonance in the side chain. The difference between the two compounds is that the methoxy group at C-25 in **1** was replaced by a hydroxy group in **2**. This substitution was suggested by the upfield shifts of C-25, from δ_C 77.0 in **1** to δ_C 73.0 in **2** (Table 1). This conclusion was further supported by 2D HMBC correlations from the proton signal at δ_H 1.26 (H_3-26)/1.29 (H_3-27) with C-25 (δ_C 73.0), as well as a 14 mass units difference between the

two molecules. The structure of **2** was additionally confirmed through 2D NMR experiments, including HSQC, HMBC, and NOESY spectra. Its relative configuration of **2** was inferred from NOESY correlations and found to be identical to that of compound **1**. Detailed examination of the NMR spectroscopic data supported the identification of **2** and in comparison with reported literature, established it as 21-*O*-methyltoosendanpentol [14]. This compound was previously reported from the leaves and stems of *Aglaia duperreana* [14] and the fruits of *Melia toosendan* [15]. Thus far, no activity was found in an inhibition assay for compound **2**.

Compound **3** was obtained as a white amorphous powder. Its molecular formula, $C_{31}H_{50}O_5$, was established based on the HRESIMS peak at *m/z* 503.3739 $[M + H]^+$ (calcd. for $[C_{31}H_{51}O_5]^+$, 503.3731) and NMR data, indicating seven degrees of unsaturation. The ^{1}H NMR spectrum of **3** showed signals corresponding to seven methyl groups, one methoxy group [δ_H 3.34 (3H, s)], three oxygenated methine protons, one olefinic proton [δ_H 5.32 (1H, dd, *J* = 6.6, 3.0 Hz, H-7], and several overlapping resonances for aliphatic methine and methylene protons. Combined analysis of the ^{13}C NMR and HSQC spectra revealed 31 carbon signals attributable to one ketone group, seven methyl groups, one methoxy carbon, eight sp^3 methylenes, seven sp^3 methines (three oxygenated at δ_C 109.0, 76.8, and 75.6), one sp^2 methine carbon (δ_C 118.2), one sp^2 non-protonated carbon (δ_C 145.6), and five sp^3 non-protonated carbons (Table 2). The remaining five degrees of unsaturation indicated that **3** possessed a pentacyclic core. The presence of a tetrahydrofuran ring in the side chain was assigned based on correlations observed in the 2D NMR data. Overall, the NMR spectroscopic data closely matched those reported for a 3-oxotirucalla-7-ene skeleton bearing a 21,23-epoxy-21-methoxy-24,25-dihydroxy side chain [16].

Table 2. NMR spectroscopic data of compounds **3** and **4** and the reference compounds

No.	$^{\#}\delta_C$ [a]	**3**		$^{\$}\delta_C$ [a]	**4**	
		δ_C [a,b]	δ_H [a,c] mult. (*J* in Hz)		δ_C [a,b]	δ_H [a,c] mult. (*J* in Hz)
1	38.5	38.5	1.47 m/1.98 m	37.2	37.2	1.14 m/1.67 m
2	35.1	35.1	2.24 dt (14.4, 3.6) 2.75 ddd (14.4, 14.4, 5.4)	27.7	27.7	1.60 m 1.64 m
3	216.9	216.8	-	79.2	79.2	3.23 m
4	47.9	47.9	-	38.9	39.0	-
5	52.3	52.4	1.72 dd (9.6, 7.8)	50.7	50.8	1.31 m
6	24.3	24.4	2.09 m/2.11 m	24.0	24.0	1.97 m/2.14 m
7	118.1	118.2	5.32 dd (6.6, 3.0)	118.2	118.2	5.26 q (3.0)
8	145.5	145.6	-	145.5	145.6	-
9	48.3	48.4	2.33 m	48.8	48.9	2.24 m
10	34.9	34.9	-	35.0	35.1	-
11	17.7	17.8	1.59 m	17.7	17.7	1.52 m
12	31.5	31.6	1.55 m/1.75 m	31.2	31.2	1.30 m/1.87 m
13	43.60	43.8	-	43.5	43.6	-
14	50.9	51.0	-	50.7	50.8	-
15	34.3	34.4	5.48 d (1.8)	34.2	34.2	1.49 m/1.54 m
16	27.4	27.4	1.75 m/1.95 m	27.3	27.3	1.29 m/1.87 m
17	50.3	50.3	1.30 m/1.94 m	45.0	45.0	2.03 t (9.0)
18	22.6	22.6	0.86 s	23.2	23.2	0.83 s
19	12.7	12.8	1.00 s	13.1	13.1	0.75 s
20	47.7	47.8	2.17 m	46.5	46.5	1.96 m
21	108.9	109.0	4.78 d (3.6)	104.8	104.9	4.68 d (3.6)
22	33.8	33.9	1.52 m	32.5	32.6	1.78 m/1.94 m
23	76.7	76.8	4.22 ddd (10.8, 5.4, 1.8)	77.8	77.8	4.44 ddd (9.6, 9.6, 2.4)
24	75.3	75.6	3.25 dd (9.6, 1.2)	76.7	77.0	3.26 dd (9.6, 2.4)
25	73.1	73.1	-	77.1	77.1	-
26	26.3	26.4	1.27 s	20.1	20.1	1.18 s
27	26.4	26.5	1.30 s	22.4	22.4	1.24 s
28	21.6	21.6	1.10 s	27.0	27.6	0.97 s
29	24.5	24.6	1.05 s	14.7	14.7	0.86 s
30	27.3	27.3	1.12 s	27.3	27.3	0.99 s
21-OCH_3	55.6	55.6	3.34 s	55.0	55.0	3.35 s
25-OCH_3				49.3	49.3	3.25 s

Measured in [a]$CDCl_3$, [b]150 MHz, and [c]600 MHz. [#]δ_C of 21α-methylmelianodiol [16] *and [$]$\delta_C$ of 21S,23R-epoxy-3β,24R-dihydroxy-21β,25-dimethoxy-tirucalla-7-en* [13].

The strong cross-peaks observed in the HMBC spectrum (Fig. 2) between the methyl protons and their neighboring carbons were used to establish the tirucallane backbone. The location of the methoxy group at C-21 was confirmed by the HMBC cross-peak from the methoxy protons to C-21. HMBC correlations from olefinic proton H-7 [δ_H 5.32 (1H, dd, J = 6.6, 3.0 Hz, H-7)] to C-5 (δ_C 52.4), and C-9 (δ_C 48.4) assigned the trisubstituted double bond to C-7 (δ_C 118.2) and C-8 (δ_C 145.6). Further structural elucidation of **3** was analyzed by $^1H-^1H$ COSY spectrum, which enabled the identification of four structural fragments: C-1−C-3, C-5−C-6−C-7, C-9−C-11−C-12, and C-15−C-16−C-17−C-20(C-21)−C-22−C-23−C-24 based on the sequences of correlated protons (Fig. 2). The NOESY cross-peaks for H-5/H-9, H-5/H_3-28, H-9/H_3-18, H_3-18/H-20, and H-20/H-23, indicated that H-5, H-9, H_3-18, H_3-28, H-23 were α-oriented. The observable NOESY correlations between H_3-29/H_3-19, H_3-30/H-17 suggested β-configurations for H-17, H_3-19, H_3-29 and H_3-30. The coupling constant between H-23 and H-24 (J = 9.6 Hz) in **3** indicated a *trans* relationship, which was further supported by the NOESY correlation between H-24/H-21. Furthermore, compound **3** displayed the same configuration at C-20 as compounds **1** and **2**. The comparison of the NMR data of **3** with NMR spectroscopic data in the published literature [16] supported the identification of **3**. Thus, compound **3** was established as 21α-methylmelianodiol, previously reported from the fruits of *Poncirus trifoliata* (Rutaceae) [16] and *Melia azedarach* [17]. 21α-Methylmelianodiol showed significant cytotoxicity against HCT116 (carcinoma) and RKO (adenocarcinoma) cell lines with IC_{50} values of 10.16 ± 1.22 and 8.57 ± 0.80 μM, respectively [17]. The anticancer potential of **3** with a redox regulatory mechanism and also outlines the mechanistic action of **3** in inhibiting the growth, survival, and metastasis of human non-small cell lung cancer cells and influences a mechanism that results in the influence on suppression of P-gp/MDR1-associated multidrug resistance in drug-resistant human non-small cell lung cancer cells [18]. Additionally, the anti-inflammatory effect of compound **3** was evaluated based on its ability to inhibit nitric oxide (NO) production in mouse peritoneal macrophages [19]. This effect was mediated through the suppression of interleukin-6 (IL-6) expression [20] and the inhibition of NF-κB phosphorylation [21].

Compound **4** was obtained as a white amorphous powder with the molecular formula $C_{32}H_{54}O_5$, as confirmed by the sodium adduct ion peak at m/z 541.3874 $[M + Na]^+$ (calcd. for $[C_{32}H_{54}NaO_5]^+$, 541.3864). The 1D and 2D NMR spectroscopic data of **4** were highly similar to those of the compound 21α-methylmelianodiol (**3**), indicating that both compounds shared the same tirucallane-type triterpenoid core [13],[22]. The obvious difference was the presence of the addition of a methoxy group (δ_H 3.25/δ_C 49.3) and a ketone signal (δ_C 216.8) in **3** instead of an oxygenated methine signal for C-3 in **4**. In the HMBC spectrum of **4**, cross-peaks from the two *gem*-dimethyl protons at δ_H 0.97 and 0.86 (each 3H, s) to the oxymethine carbon resonance δ_C 79.2 suggested the presence of a hydroxy group at the C-3 position. Furthermore, the methoxy group was assigned to C-25 based on the HMBC correlation between OMe (δ_H 3.25) and C-25 (δ_C 77.1). The configuration of the hydroxy group was determined to be β-orientation from the multiplicity of H-3 (δ_H 3.23, m), in contrast to the broad singlet typically observed for an α-orientation [13],[22]. Similarly, a β-orientation of the methoxy group at C-21 was supported by the ^{13}C NMR chemical shift of C-21 in **4** (δ_C 104.9), which closely matched the corresponding value reported for 21*S*,23*R*-epoxy-3β,24*R*-dihydroxy-21β,25-dimethoxy-tirucalla-7-en [13] at δ_C 104.8. A comprehensive analysis of the NMR spectroscopic data, together with comparison to previously published data [13],[22], led to the identification of **4** to be 21*S*,23*R*-epoxy-3β,24*R*-dihydroxy-21β,25-dimethoxy-tirucalla-7-en.
Compound **4** was evaluated for its growth-inhibitory effects on HepG2 (liver), K562 (leukemia), SGC7901 (gastric), and HL60 (leukemia) cancer cell lines using MTT and SRB assays. The results showed that compound **4** exhibited moderate cytotoxic activity against the HL60 cell line, with an IC_{50} value of 44.9 μM, in comparison with cisplatin (IC_{50} = 4.2 μM) [13].

Two remaining compounds were determined to be odoratone (**5**) [23] and cabraleone (**6**) [24] by comparison of their physical and spectroscopic data to those reported in the references. Indeed, odoratone (**5**) was reported for the first time by Chan and co-workers in 1967 from *Cedrela odorata* based on its IR absorption bands and the characteristic proton signals [25].

Its ^{13}C NMR data were published for the first time by Luo et al [23]. Unfortunately, although compounds **5** and **6** have been reported in many other plants, it is worth noting that there is still little research on their biological assay.

4. Conclusions

The chemical constituents of *W. pinnata* leaves have been identified in the present study. Six compounds were isolated, including cumingianol E (**1**), 21-*O*-methyltoosendanpentol (**2**), 21α-methylmelianodiol (**3**), 21*S*,23*R*-epoxy-3β,24*R*-dihydroxy-21β,25-dimethoxy-tirucalla-7-en (**4**), odoratone (**5**), and cabraleone (**6**). The structures of these compounds were elucidated through NMR spectroscopic analysis and comparison with previously reported data. To the best of our knowledge, the occurrence of compounds **1**−**6** are reported here for the first time in this species.

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