TRITERPENOIDS FROM *DISCHIDIA ALBOFLAVA* WHOLE PLANT

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Abstract - In this report, phytochemical constituents of *Dischidia alboflava* (Asclepiadaceae) collected in Bidoup Nui Ba National Park, Lamdong province, Vietnam were investigated for the first time. The isolation process, as well as structure elucidation, have been supported by modern techniques like chromatographic (TLC, CC) and spectroscopic (ESI MS, 1D and 2D NMR) methods to result six triterpenoids, including *β*-amirin acetate (**1**), friedelin (**2**), 3*α*-hydroxyglutin-5-en (**3**), lupeone (**4**), lupeol (**5**), and 30-norcyclopterospemol (**6**). Among these, compounds **3**, **4**, and **6** have not been recorded to occur in other *Dischidia* species before. Notably, the identification of compound **6** indicated the presence of cyclotriterpene in *Dischidia* chemical components, which has not been reported to date.

Key words - Asclepiadaceae; cyclotriterpene; *Dischidia alboflava*; triterpenoids

1. Introduction

Dischidia, a small genus of the family Asclepiadaceae, consists of about 80 herbs or small shrubs mainly distributed in Asia like Indochina, the East Pacific [1]. Plants belonging to *Dischidia* genus were renowned in the traditional medicinal system to cure respiratory diseases, symptoms of urinary cystitis, inflammation, leucorrhoea, tumor and cancer [2]. Extensive use of *Dischidia* plants in traditional medicinal system need to be proved by phytochemical studies, as well as pharmacological investigations. However, the number of studies is still very limited. Up to now, just only five *Dischidia* species (*D. acuminata*, *D. chinensis*, *D. formosana*, *D. imbricate* and *D. nummularia*) had been studied for phytochemistry to show the presence of some alkaloids, flavonoids, mono-phenols, fatty acids, steroids, and terpenoids [2, 3-5].

Being one of twelve *Dischidia* species found out in Vietnam [1], *Dischidia alboflava* Cost. has been scientifically described in 2012 as a flowering vine [6]. However, to date, there are no studies analyzing the chemical constituents of this plant. All above-mentioned factors prompted us to carry out the chemical investigation of *D. alboflava*, resulting in the isolation of six triterpenoids (**1-6**), which fell into different subgroups of oleanane, friedelane, glutinane, lupane, and cyclotriterpene.

2. Materials and methods

2.1. Plant materials

The whole plant of *D. alboflava* was collected in Bidoup Nui Ba National Park, Lamdong province, Vietnam in December 2020. The plant was taxonomically identified by taxonomist Dr. Dang Viet Hung, Vietnam National University of Forestry at Dongnai, Dongnai, Vietnam. A voucher specimen (VHH.LD.12.2020.1) was stored at the Institute of Chemistry, VAST, Ha Noi.

2.2. General experimental procedures

ESI MS spectrum was obtained on Agilent LC-MSD-Trap SL (Applied Biosystems). ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) spectra were recorded on a Bruker Avance 600 FT-NMR spectrometer at 25°C. The spectra were run as $CDCl_3$ solution and were referenced to as solutions for the internal standard (^{1}H δ 7.26; ^{13}C δ 77.2). Coupling constants were reported in Hertz (Hz). Silica gel 60 F-254 (0.25mm, Merck). CC: Silica gel 60 (230-400 mesh, Merck), silica gel 60, 40-63 μm (Merck) and Sephadex LH-20.

2.3. Extraction and isolation

The whole plant of *D. alboflava* was dried and powdered to give the sample of 4.5 kg, which then was extracted three times with MeOH (95%) at room temperature, each time overnight (24 hours). MeOH extracts were combined, and the solvent was evaporated *in vacuo* at 40^{0}C to obtain aqueous solution, which was then partitioned with *n*-hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and *n*-butanol (three times for each). The organic solvents were evaporated to yield *n*-hexane (120 g), CH_2Cl_2 (27.8 g), EtOAc (83.9 g) and *n*-butanol (225 g) extracts, respectively.

The *n*-hexane extract (120 g) was separated on column chromatography using silica gel as absorbent and eluting with gradient solvent system of *n*-hexane:acetone (95:5 → 60:40) to give 5 fractions (H1 – H5). Fr. H1 (6.3 g) was subjected on silica gel column chromatography (CC) with gradient elution system of *n*-hexane:CH_2Cl_2 to give 8 subfractions (H1.1 – H1.8). Compounds **1** (10 mg) and **2** (7 mg) were obtained by separating H1.1 (0.8 g) on a silica gel column chromatography, eluted with *n*-hexane:CH_2Cl_2 (80:20). The subfraction H1.3 (1.2 g) was chromatographed by using a silica gel column and solvent system (*n*-hexane:CH_2Cl_2, 5 → 30% CH_2Cl_2) to yield compounds **3** (6 mg) and **6** (8 mg). Compounds **4** (5 mg), and **5** (12 mg) were isolated from subfraction H1.8 (0.9 g) by rechromatography on silica gel eluting with *n*-hexane:CH_2Cl_2 (70:30).

β-Amyrin acetate (**1**): White powder; ESI-MS: *m/z* = 469.41 $[M+H]^+$; ^{1}H-NMR (600 MHz, $CDCl_3$): 1.55 (m, H-1a), 1.49 (m, H-1b), 1.61 (m, H-2a), 1.52 (m, H-2b), 4.50 (dd, 5.0, 8.0, H-3), 0.81 (m, H-5), 1.51 (m, H-6a), 1.34 (m, H-6b), 1.58 (m, H-7a), 1.51 (m, H-7b), 1.54 (m, H-9), 1.89 (m, H-11), 5.18 (t, 3.0, H-12), 1.50 (m, H-15a), 1.44 (m, H-15b), 1.31 (m, H-16a), 1.52 (m, H-16b), 1.29 (m, H-18), 1.38 (m, H-19a), 1.98 (m, H-19b), 1.66 (m, H-21), 1.52 (m, H-22), 0.875 (s, H-23), 0.875 (s, H-24), 0.97 (s, H-25), 0.96 (s, H-26), 1.13 (s, H-27), 0.83 (s, H-28), 0.86 (s, H-29), 0.86 (s, H-30), 2.04 (s, $\underline{C}H_3COO$); ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1.

Friedelin (**2**): White powder; ESI-MS: *m/z* = 427.40 $[M+H]^+$; ^{1}H-NMR (600 MHz, $CDCl_3$): 1.95 (m, H-1a), 1.71 (m, H-1b), 2.37 (H-2a), 2.27 (H-2b), 2.25 (q, 6.6, H-4), 1.74 (m, H-6a), 1.28 (m, H-6b), 1.49 (m, H-7a), 1.36 (m, H-7b), 1.38 (m, H-8), 1.53 (m, H-10), 1.45 (m, H-11a), 1.36 (m, H-11b), 1.33 (m, H-12a), 1.32 (m, H-12b), 1.47 (m, H-15a), 1.27 (m, H-15b), 1.58 (m, H-16a), 1.35 (m, H-16b), 1.56 (m, H-18), 1.37 (m, H-19a), 1.22 (m, H-19b), 1.50 (m, H-21a), 1.31 (m, H-21b), 1.51 (m, H-22a), 0.95 (m, H-22b), 0.88 (d, 7.2, H-23), 0.73 (s, H-24), 0.87 (s, H-25), 1.01 (s, H-26), 1.05 (s, H-27), 1.18 (s, H-28), 1.00 (s, H-29), 0.95 (s, H-30); ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1.

3*α*-Hydroxyglutin-5-en (**3**): White powder; ESI-MS: *m/z* = 427.42 $[M+H]^+$; ^{1}H-NMR (600 MHz, $CDCl_3$): 1.48 (m, H-1a), 1.54 (m, H-1b), 1.69 (m, H-2a), 1.65 (m, H-2b), 3.47 (brs, H-3), 5.62 (d, 6.0, H-6), 1.84 (m, H-7a), 1.97 (m, H-7b), 1.51 (m, H-8), 2.01 (m, H-10), 1.39 (m, H-11a), 1.54 (m, H-11b), 1.35 (m, H-12), 1.30 (m, H-15a), 1.47 (m, H-15b), 1.39 (m, H-16a), 1.54 (m, H-16b), 1.60 (m, H-18), 1.25 (m, H-19a), 1.39 (m, H-19b), 1.25 (m, H-21a), 1.47 (m, H-21b), 0.92 (m, H-22a), 1.54 (m, H-22b), 1.15 (s, H-23), 1.12 (s, H-24), 1.08 (s, H-25), 1.03 (s, H-26), 0.99 (s, H-27), 0.97 (s, H-28), 0.94 (s, H-29), 0.83 (s, H-30); ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1.

Lupeone (**4**): White powder; ESI-MS: *m/z* = 425.37 $[M+H]^+$; ^{1}H-NMR (600 MHz, $CDCl_3$): 1.92 (m, H-1a), 1.42 (m, H-1b), 2.48 (m, H-2a), 2.38 (m, H-2b), 1.29 (m, H-5), 1.47 (m, H-6), 1.42 (m, H-7a), 1.25 (m, H-7b), 1.31 (m, H-9), 1.65 (m, H-11a), 1.51 (m, H-11b), 1.47 (m, H-12), 1.38 (m, H-13), 1.49 (m, H-15a), 1.29 (m, H-15b), 1.51 (m, H-16a), 1.48 (m, H-16b), 1.39 (m, H-18), 1.89-1.95 (m, H-1, H-21), 2.40 (m, H-2, H-19), 1.62 (m, H-22a), 1.07 (s, H-23), 1.03 (s, H-24), 0.93 (s, H-25), 1.07 (s, H-26), 0.96 (s, H-27), 0.80 (s, H-28), 4.57 (brs, H-29a), 4.69 (brs, H-29b), 1.68 (s, H-30).

Lupeol (**5**): white needles; ESI-MS: *m/z* = 427.39 $[M+H]^+$; ^{1}H (600 MHz, $CDCl_3$): 1.65 (m, H-1a), 0.92 (m, H-1b), 1.62 (m, H-2), 3.19 (dd, 4.0, 9.0, H-3), 0.67 (t, 9.5, H-5), 1.38 (m, H-6), 1.52 (m, H-7a), 1.35 (m, H-7b), 1.25 (m, H-9), 1.38 (m, H-11a), 1.18 (m, H-11b), 1.60 (m, H-12a), 1.01 (H-12b), 2.11 (m, H-13), 1.61 (m, H-15a), 1,01 (m, H-15b), 2.11 (m, H-16a), 1.25 (H-16b), 1.35 (m, H-18), 2.36 (H-19), 1.86 (m, H-21), 1.27 (m, H-22), 0.97 (s, H-23), 0.76 (s, H-24), 0.83 (s, H-25), 1.03 (s, H-26), 0.95 (s, H-27), 0.79 (s, H-28), 4.57 (brs, H-29a), 4.68 (brs, H-29b), 1.68 (s, H-30).

30-Norcyclopterospemol (**6**): Colorless crystal; ESI-MS: *m/z* = 427.39 $[M+H]^+$; ^{1}H-NMR (600 MHz, $CDCl_3$): 1.53 (m, H-1a), 1.25 (m, H-1b), 2.00 (m, H-2a), 1.41 (m, H-2b), 3.2 (m, H-3), 1.16 (m, H-4), 1.20 (m, H-5), 1.61 (m, H-6a), 0.56 (m, H-6b), 1,95 (m, H-7a), 1.35 (m, H-7b), 1.35 (m, H-11a), 1.10 (m, H-11b), 1.51 (m, H-12a), 1.15 (m, H-12b), 1.52 (m, H-15), 1.95 (m, H-16a), 1.25 (m, H-16b), 1.51 (m, H-17), 1.04 (s, H-18), 0.39 (d, 3.0, H-19a), 0.60 (d, H-19b), 1.49 (m, H-20), 1.05 (d, 3.0, H-21), 1.52 (m, H-23), 1.30 (m, H-24), 1.75 (m, H-25), 1.03 (d, 3.0, H-26), 1.03 (d, 3.0, H-27), 0.89 (s, H-28), 0.96 (d, 7.2, H-29), 4.72 (brs, H-30a), 4.67 (brs, H-30b); ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1.

Table 1. *^{13}C NMR data [150 MHz, $CDCl_3$, δppm] of compounds **1-3**, and **6***

C	1	2	3	6
1	38.3 CH_2	22.3 CH_2	18.2 CH_2	30.8 CH_2
2	28.0 CH_2	41.5 CH_2	27.8 CH_2	34.8 CH_2
3	81.0 CH	213.3 C	76.4 CH	76.6 CH
4	37.7 C	58.2 CH	40.8 C	44.6 CH
5	55.3 CH	42.2 C	141.6 C	43.4 CH
6	18.3 CH_2	41.3 CH_2	122.1 CH	24.7 CH_2
7	33.3 CH_2	18.2 CH_2	23.6 CH_2	28.1 CH_2
8	39.8 C	53.1 CH	47.4 CH	46.9 C
9	47.6 CH	37.4 C	34.9 C	23.6 C
10	36.8 C	59.5 CH	49.7 CH	29.6 C
11	23.5 CH_2	35.4 CH_2	33.1 CH_2	25.2 CH_2
12	121.6 CH	30.5 CH_2	30.4 CH_2	35.0 CH_2
13	145.2 C	39.7 C	37.8 C	45.4 C
14	41.7 C	38.3 C	39.3 C	48.9 C
15	26.1 CH_2	32.4 CH_2	34.6 CH_2	32.9 CH_2
16	26.9 CH_2	36.0 CH_2	35.1 CH_2	27.0 CH_2
17	32.6 C	30.0 C	30.1 C	52.2 CH
18	47.2 CH	42.8 CH	43.1 CH	17.8 CH_3
19	46.8 CH_2	35.6 CH_2	36.0 CH_2	27.2 CH_3
20	31.1 C	28.2 C	28.3 C	36.1 CH
21	34.7 CH_2	32.8 CH_2	32.1 CH_2	18.4 CH_3
22	37.1 CH_2	39.3 CH_2	39.0 CH_2	156.9 C
23	28.4 CH_3	6.8 CH_3	29.0 CH_3	33.8 CH_2
24	15.6 CH_3	14.7 CH_3	25.5 CH_3	35.4 CH_2
25	16.7 CH_3	17.9 CH_3	16.2 CH_3	31.3 CH
26	16.8 CH_3	20.3 CH_3	18.4 CH_3	21.9 CH_3
27	25.9 CH_3	18.7 CH_3	19.6 CH_3	22.0 CH_3
28	28.4 CH_3	32.1 CH_3	32.4 CH_3	19.1 CH_3
29	32.5 CH_3	35.0 CH_3	32.0 CH_3	14.4 CH_3
30	23.7 CH_3	31.7 CH_3	34.5 CH_3	106.0 CH_2
$CH_3\underline{C}OO$	171.0 C	-	-	-
$\underline{C}H_3COO$	21.3 CH_3	-	-	-

3. Results and discussions

The chemical structures of six isolated triterpenoids (**1**-**6**) (Figure 1) were established by using spectroscopic analysis including mass spectrum (ESI MS) and NMR spectroscopic data as well as by comparing their spectroscopic data with those of reported literature.

The molecular formula of compound **1** was determined to be $C_{32}H_{52}O_2$ from the analysis of its mass spectrum ESI-MS: *m/z* = 469.41$[M+H]^+$ and 1D-NMR spectroscopic data. The ^{1}H- and ^{13}C NMR spectra of **1** showed resonance characteristics of a triterpene with oleanane skeleton [7]. This phenomenon was explained

based on the presence of eight singlet methyls at H-23 (δ_H 0.88)/C-23 (δ_C 28.4), H-24 (δ_H 0.88)/C-24 (δ_C 15.6), H-25 (δ_H 0.97)/C-25 (δ_C 16.7), H-26 (δ_H 0.96)/C-26 (δ_C 16.8), H-27 (δ_H 1.13)/C-27 (δ_C 25.9), H-28 (δH 0.83)/C-28 (δ_C 28.4), H-29 (δ_H 0.87)/C-29 (δ_C 32.5), and H-30 (δ_H 0.86)/C-30 (δ_C 23.7). In addition, the double-bond at C-12 position was determined by proton signal at δ_H 5.18 (t, 3.0, H-12) and two downfield carbons at δ_C 121.6 (C-12) and 145.2 C-13). Notably, the hydroxyl group at C-3 was acetylated identifying via the downfield oxymethine signal at δ_H 2.04/δ_C 21.3, and the acetyl group at δ_C 171.0 ppm. The *β*-configuration of acetyl group at C-3 position was suggested via the large coupling constant at δ_H 4.5 (dd, 5.0, 8.0, H-3) [7]. The spectral data of **1** was consistent with those reported in previous studies for *β*-amyrin acetate [8]. The isolation of **1** was once again confirmed by the existence of oleanane-triterpene in the genus *Dischidia*.

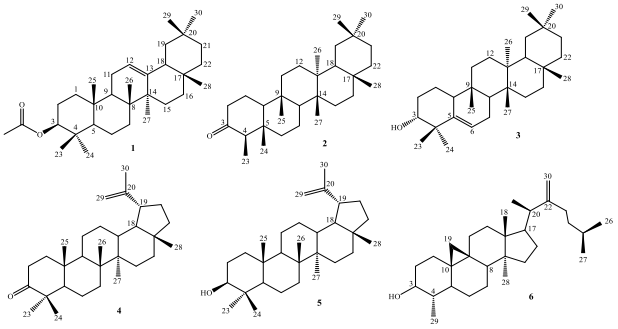

Figure 1. *Structures of compounds **1-6** isolated from D. alboflava*

Compound **2** was isolated and identified to have the molecular formula of $C_{30}H_{50}O$ based on the ESI MS peak at *m/z* 427.40 $[M+H]^+$. The 30 typical carbon signals together with the methyl proton signal of C-23 position at δ_H 0.88 (d, 7.2, H-23) appeared as a doublet as expected for a friedelane triterpene [9]. Apart from that, its ^{1}H-NMR spectra gave one doublet methyl at δ_H 0.88 (d, 7.2, H-23) and seven singlet methyls at δ_H 0.73 (s, H-24), 0.87 (s, H-25), 1.01 (s, H-26), 1.05 (s, H-27), 1.18 (s, H-28), 1.00 (s, H-29), and 0.95 (s, H-30). There was a spectrum at a chemical shift between δ_H1.3 to 2.5 ppm indicating the presence of methylene and methine groups (CH_2 and CH). In the ^{13}C-NMR spectrum of the isolate, showed the presence of 8 methyl groups at chemical shifts δ_C 6.8, 14.7, 17.9, 18.7, 20.3, 31.7, 32.1, and 35.0 ppm. The presence of a carbonyl group was indicated by the signal at δ_C 213.3 (C-3) in ^{13}C-NMR spectra. All these observations were in agreement with triterpene friedelin, which has been found in *D. formosana* in 1993 [3].

Compound **3** was obtained as a white powder. Molecular formula of **3** was identified as $C_{30}H_{50}O$ based on the 1D-NMR spectroscopic data and the ESI-MS data *m/z* 427.42 $[M+H]^+$. ^{1}H- and ^{13}C-NMR spectra of **3** indicated the typical characters of a glutinane-type triterpenoid when showed the signals of 30 carbons in ^{13}C-NMR spectra, and eight methyl groups of singlet signals were clearly detected at δ_H 1.15 (s, H-23), 1.12 (s, H-24), 1.08 (s, H-25), 1.03 (s, H-26), 0.99 (s, H-27), 0.97 (s, H-28), 0.94 (s, H-29), and 0.83 (s, H-30) in ^{1}H-NMR spectra. The presence of a double bond at C-5/C-6 were suggested via signals at δ_C141.6 and 122.1 corresponding to olefinic proton at δ_H 5.62 (d, 6.0, H-6). In addition, the signal at δ_H 3.47 (brs, H-3) indicated the presence of a hydroxyl group with *α*-configuration at C-3 position (δ_C 76.4). Therefore, compound **3** was determined to be 3*α*-hydroxyglutin-5-ene when compared spectroscopic data to its 3-*β*-epimer published in the literature [10]. As the results of literature search, compound **3** was reported herein for the first time in *Dischidia* species.

Two compounds **4** and **5** were purified as white needles. Their molecular formulas were identified as $C_{30}H_{48}O$ and $C_{30}H_{50}O$ basing on respective ESI-MS signals at *m/z* 424.37 and 427.39 $[M+H]^+$. Both these two compounds charactered triterpenoids of lupane skeleton when contained seven singlet methyl ranged from δ_H 0.76 to 1.68 ppm, one olefinic group H-29 (δ_H 4.57 and 4.69 ppm), and the remaining aliphatic methines and methylenes (δ_H 1.20 to 2.60 ppm). Basing on the NMR data and comparison with the data in published literature, compound **4** was elucidated as lupeone [11]. The NMR data of **5** are very similar to those of **4**, except for carbonyl group at C-3 in compound **4** was replaced by one hydroxyl group in compound **5**. Hydroxygenation occurred at carbon C-3 of **5** in *β*-configuration was demonstrated based on the presence of downfield oxy-methine proton at δ_H 3.19 (dd, 4.0, 9.0, H-3) [12]. In comparison with literature data, compound **5** was elucidated as a renowned triterpenoid, namely lupeol [13]. Lupeol (**5**) has been isolated from other *Dischidia* species before [5], but this is the first time lupeone (**4**) was reported from this genus.

Compound **6** was obtained as a colorless crystal with the molecular formula of $C_{30}H_{50}O$, which has been established based on 1D-, 2D-NMR spectroscopic analyses and ESI-MS (*m/z* 427.39 $[M+H]^+$). 30 carbon signals in ^{13}C-NMR spectra of **6** suggested a triterpenoid structure. The presence of a cyclopropane ring was identified from the methylene carbon at δ_C 27.2 (C-19) corresponding to δ_H 0.39 (d, 3.0, H-19a) and 0.60 (d, H-19b) based on HSQC spectra analysis, together with six methyl groups, including two singlet and four doublet at δ_H 1.04 (s, H-18), 1.05 (d, 3.0, H-21), 1.03 (d, 3.0, H-26), 1.03 (d, 3.0, H-27), 0.89 (s, H-28), and 0.96 (d, 7.2, H-29) were found out in ^{1}H-NMR spectra suggested a cyclolanostane skeleton of **6**. Apart from that, one hydroxyl group was attached to carbon C-3 and one methylene group to C-22 were demonstrated by the the presence of methine carbon signals at δ_C 76.6 (C-3) and quaternary carbon at δ_C 156.9 (C-22), respectively. These suggestions were one again confirmed by oxy-methine proton at δ_H 3.2 (m, H-3) and methylene proton at δ_H 4.72 (brs, H-30a), 4.67 (brs, H-30b) in ^{1}H-NMR spectra. The structure of **6** was therefore established as 30-nor-22-methylene-9,19-cyclolanostan-3-ol (or 30-norcyclopterospermol), which has been reported as a new compound from *Pterospermum heyneanum* before [14]. 30-norcyclopterospermol (**6**) was considered to be the first representative of cyclotriterpene group in *Dischidia* species and up to now there is no biological study conducted on this compound.

4. Conclusion

In conclusion, we here reported for the first time the chemical constituents of *D. alboflava* collected in Bidoup Nui Ba National Park, Lamdong province, Vietnam. The results showed that six triterpenoids have been isolated and identified from the whole plant of *D. alboflava*. It is intriguing to point out that *D. alboflava* is distinctive for being one out of 80 *Dischidia* species to contain cyclotriterpene. Therefore, cyclotriterpene **6** serves as a valuable chemotaxonomic marker of *D. alboflava* from *Dischidia* plants.

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